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(54) Title: VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS

(57) Abstract

A biosynthetic method for producing vitamin C (ascorbic acid, L–ascorbic acid, or AA) is disclosed. Such a method includes fermentation of a genetically modified microorganism or plant to produce L–ascorbic acid. In particular, the present invention relates to the use of microorganisms and plants having at least one genetic modification to increase the action of an enzyme involved in the ascorbic acid biosynthetic pathway. Included is the use of nucleotide sequences encoding epimerases, including the endogenous GDP–D–mannose:GDP–L–galactose epimerase from the L–ascorbic acid pathway and homologues thereof for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L–ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

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VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS

FIELD OF THE INVENTION

The present invention relates to vitamin C (L-ascorbic acid) production using genetically modified microorganisms and plants. In particular, the present invention relates to the use of nucleotide sugar epimerase enzymes for the biological production of ascorbic acid in plants and microorganisms.

BACKGROUND OF THE INVENTION

Nearly all forms of life, both plant and animal, either synthesize ascorbic acid (vitamin C) or require it as a nutrient. Ascorbic acid was first identified to be useful as a dietary supplement for humans and animals for the prevention of scurvy. Ascorbic acid, however, also affects human physiological functions such as the adsorption of iron, cold tolerance, the maintenance of the adrenal cortex, wound healing, the synthesis of polysaccharides and collagen, the formation of cartilage, dentine, bone and teeth, the maintenance of capillaries, and is useful as an antioxidant.

For use as a dietary supplement, ascorbic acid can be isolated from natural sources, such as rosehips, synthesized chemically through the oxidation of L-sorbose, or produced by the oxidative fermentation of calcium D-gluconate by *Acetobacter suboxidans*. Considine, "Ascorbic Acid," *Van Nostrand's Scientific Encyclopedia*, Vol. 1, pp. 237-238, (1989). Ascorbic acid (predominantly intracellular) has also been obtained through the fermentation of strains of the microalga, *Chlorella pyrenoidosa*. See U.S. Patent No. 5,001,059 by Skàtrud, which is assigned to the assignee of the present application. It is believed that ascorbic acid is produced inside the chloroplasts of photosynthetic microorganisms and functions to neutralize energetic electrons produced during photosynthesis. Accordingly, ascorbic acid production is known in photosynthetic organisms as a protective mechanism.

Therefore, products and processes which improve the ability to biosynthetically produce ascorbic acid are desirable and beneficial for the improvement of human health.

SUMMARY OF THE INVENTION

One embodiment of the present invention relates to a method for producing ascorbic acid or esters thereof in a microorganism. The method includes the steps of: (a)

culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase; and (b) recovering the ascorbic acid or esters produced by the microorganism. Preferably, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In one embodiment of the method of the present invention, the microorganism further includes a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase. Such a genetic modification can include, for example, a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

In one embodiment, the genetic modification is a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, which can include GDP-D-mannose:GDP-L-galactose epimerase. In one embodiment, the epimerase binds NADPH. In one embodiment of this method, the genetic modification includes transformation of the microorganism with a recombinant nucleic acid molecule that expresses the epimerase. Such an epimerase can have a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the epimerase has a structure having an average root mean square deviation of less than about 2.5 Å, and more preferably less than about 1 Å, over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In one embodiment, the epimerase comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by

atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Such a substrate binding site preferably has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In another embodiment, the epimerase comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Such a catalytic site preferably has a tertiary structure with an average root mean square deviation of less than about 1 Å over at least about 25% of Cα positions of the tertiary structure of a catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. The catalytic site preferably includes the amino acid residues serine, tyrosine and lysine and in one embodiment, the tertiary structure positions of the amino acid residues serine, tyrosine and lysine substantially conform to tertiary structure positions of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws.

In yet another embodiment of this method, the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50%, and in another embodiment with at least about 75%, and in yet another embodiment with at least about 90% of non-Xaa residues in SEQ ID NO:11. In another embodiment, the epimerase comprises an amino acid sequence having at least 4 contiguous amino acid residues that are 100% identical to at least 4 contiguous amino acid residues of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. In yet another embodiment, the recombinant nucleic acid molecule comprises a nucleic acid sequence comprising at least about 12 contiguous nucleotides having 100% identity with at least about 12

contiguous nucleotides of a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

In yet another embodiment of this method of the present invention, the epimerase comprises an amino acid sequence having a motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly. In yet another embodiment, the recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 15% identical, and in another embodiment, at least about 20% identical, and in another embodiment, at least about 25% identical, to a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

In yet another embodiment of this method of the present invention, the recombinant nucleic acid molecule comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase. The nucleic acid sequence encoding the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase includes nucleic acid sequences selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, and the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can include an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

In one embodiment of the method of the present invention, the microorganism is selected from the group of bacteria, fungi and microalgae. In one embodiment, the microorganism is acid-tolerant. Preferred bacteria include, but are not limited to *Azotobacter* and *Pseudomonas*. Preferred fungi include, but are not limited to, yeast, including, but not limited to *Saccharomyces* yeast. Preferred microalgae include, but are not limited to, microalgae of the genera *Prototheca* and *Chlorella*, with microalgae of the genus *Prototheca* being particularly preferred.

In yet another embodiment of the method of the present invention, the microorganism is acid-tolerant and the step of culturing is conducted at a pH of less than about 6.0, and more preferably, at a pH of less than about 5.5, and even more preferably, at a pH of less than about 5.0. The step of culturing can be conducted in a fermentation medium that comprises a carbon source other than D-mannose in one embodiment, and

in another embodiment, the step of culturing is conducted in a fermentation medium that comprises glucose as a carbon source.

In yet another embodiment of the present method, the step of culturing is conducted in a fermentation medium that is magnesium (Mg) limited. Preferably, the step of culturing is conducted in a fermentation medium that is Mg limited during a cell growth phase. In one embodiment, the fermentation medium includes less than about 0.5 g/L of Mg during a cell growth phase, and more preferably, less than about 0.2 g/L of Mg during a cell growth phase, and even more preferably, less than about 0.1 g/L of Mg during a cell growth phase.

Another embodiment of the present invention relates to a microorganism for producing ascorbic acid or esters thereof. The microorganism has a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. Preferably, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase, and even more preferably, to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

In one embodiment, the microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In another embodiment, the microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a

CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11. Preferred microorganisms are disclosed as for the method discussed above.

Yet another embodiment of the present invention relates to a plant for producing ascorbic acid or esters thereof. Such a plant has a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In a preferred embodiment, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase, and in a more preferred embodiment, the genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

In one embodiment, the plant further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase. Such a genetic modification includes a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase. Such a plant also includes a plant that has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In another embodiment, such a plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

In one embodiment, a plant for producing ascorbic acid or esters thereof according to the present invention is a microalga. Preferred microalgae include, but are not limited to microalgae of the genera *Prototheca* and *Chlorella*, with microalga of the genus *Prototheca* being particularly preferred. In another embodiment, the plant is a higher plant, with consumable higher plants being more preferred.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A is a schematic drawing of the pathway from glucose to GDP-D-mannose in plants.

Fig. 1B is a schematic drawing of the pathway from GDP-D-mannose to L-galactose-1-phosphate in plants.

Fig. 1C is a schematic drawing of the pathway from L-galactose to L-ascorbic acid in plants.

Fig. 2A is a schematic drawing of selected carbon flow from glucose in *Prototheca*.

Fig. 2B is a schematic drawing of selected carbon flow from glucose in *Prototheca*.

Fig. 3 is a schematic drawing that shows the lineage of mutants derived from *Prototheca moriformis* ATCC 75669, and their ability to produce L-ascorbic acid.

Fig. 4 is a bar graph illustrating the conversion of substrates by resting cells of strain NA45-3 following growth in media containing various magnesium concentrations and resuspension in media containing various magnesium concentrations.

Fig. 5 is a line graph showing the relationship between specific ascorbic acid formation in cultures of *Prototheca* strains and the specific activity of GDP-D-mannose:GDP-L-galactose epimerase in extracts prepared from cells harvested from the same cultures.

Fig. 6 is a line graph showing the relationship between specific epimerase activity and the degree of magnesium limitation in two strains, ATCC 75669 and EMS13-4.

Fig. 7 depicts the overall catalytic mechanism of GDP-D-mannose:GDP-L-galactose epimerase proposed by Barber (1979, *J. Biol. Chem.* 254:7600-7603).

Fig. 8A depicts the catalytic mechanism of GDP-D-mannose-4,6-dehydratase (converts GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose).

Fig. 8B depicts the catalytic mechanism of GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (converts GDP-4-keto-6-deoxy-D-mannose to GDP-L-fucose) (Chang, et al., 1988, *J. Biol. Chem.* 263:1693-1697; Barber, 1980, *Plant Physiol.* 66:326-329).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a biosynthetic method and production microorganisms and plants for producing vitamin C (ascorbic acid, L-ascorbic acid, or AA). Such a method includes fermentation of a genetically modified microorganism to produce L-ascorbic acid. In particular, the present invention relates to the use of nucleotide sequences encoding epimerases, including the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway, as well as epimerases having structural homology (e.g., by nucleotide/amino acid sequence and/or tertiary structure of the encoded protein) to GDP-4-keto-6-deoxy-D-mannose epimerase/reductases, or UDP-galactose 4-epimerases, for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L-ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

One embodiment of the present invention relates to a method to produce L-ascorbic acid by fermentation of a genetically modified microorganism. This method includes the steps of (a) culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase; and (b) recovering L-ascorbic acid or esters thereof. The various enzymes in this list represent the enzymes involved in the vitamin C biosynthetic pathway in plants. It is uncertain at this time

whether the enzyme represented by GDP-L-galactose phosphorylase is actually a phosphorylase or a pyrophosphorylase (i.e., GDP-L-galactose pyrophosphorylase). Therefore, use of the term "GDP-L-galactose phosphorylase" herein refers to either GDP-L-galactose phosphorylase or GDP-L-galactose pyrophosphorylase. In one aspect of the invention, this method includes the step of culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. This aspect of the present invention is discussed in detail below.

Another embodiment of the present invention relates to a genetically modified microorganism for producing L-ascorbic acid or esters thereof. Another embodiment of the present invention relates to a genetically modified plant for producing L-ascorbic acid or esters thereof. Both genetically modified microorganisms (e.g., bacteria, yeast, microalgae) and plants (e.g., higher plants, microalgae) have a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In a preferred embodiment, both genetically modified microorganisms (e.g., bacteria, yeast, microalgae) and plants (e.g., higher plants, microalgae) have a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. In one embodiment, the genetic modification includes the transformation of the microorganism or plant with the epimerase as described above.

To produce significantly high yields of L-ascorbic acid by the method of the present invention, a plant and/or microorganism is genetically modified to enhance production of L-ascorbic acid. As used herein, a genetically modified plant (such as a higher plant or microalgae) or microorganism, such as a microalga (*Prototheca, Chlorella*), *Escherichia coli*, or a yeast, is modified (i.e., mutated or changed) within its genome and/or by recombinant technology (i.e., genetic engineering) from its normal (i.e., wild-type or naturally occurring) form. In a preferred embodiment, a genetically modified plant or microorganism according to the present invention has been modified by

recombinant technology. Genetic modification of a plant or microorganism can be accomplished using classical strain development and/or molecular genetic techniques, include genetic engineering techniques. Such techniques are generally disclosed herein and are additionally disclosed, for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Roessler, 1995, *Plant Lipid Metabolism*, pp. 46-48; and Roessler et al., 1994, in Bioconversion for Fuels, Himmel et al. eds., American Chemical Society, Washington D.C., pp 255-70). These references are incorporated by reference herein in their entirety.

In some embodiments, a genetically modified plant or microorganism can include a natural genetic variant as well as a plant or microorganism in which nucleic acid molecules have been inserted, deleted or modified, including by mutation of endogenous genes (e.g., by insertion, deletion, substitution, and/or inversion of nucleotides), in such a manner that the modifications provide the desired effect within the plant r microorganism. As discussed above, a genetically modified plant or microorganism includes a plant or microorganism that has been modified using recombinant technology.

As used herein, genetic modifications which result in a decrease in gene expression, an increase in inhibition of gene expression or inhibition of a gene product (i.e., the protein encoded by the gene), a decrease in the function of the gene, or a decrease in the function of the gene product can be referred to as inactivation (complete or partial), deletion, interruption, blockage, down-regulation, or decreased action of a gene. For example, a genetic modification in a gene which results in a decrease in the function of the protein encoded by such gene can be the result of a complete deletion of the gene encoding the protein (i.e., the gene does not exist, and therefore the protein does not exist), a mutation in the gene encoding the protein which results in incomplete or no translation of the protein (e.g., the protein is not expressed), or a mutation in the gene which decreases or abolishes the natural function of the protein (e.g., a protein is expressed which has decreased or no enzymatic activity).

Genetic modifications which result in an increase in gene expression or function can be referred to as amplification, overproduction, overexpression, activation, enhancement, addition, up-regulation or increased action of a gene. Additionally, a genetic modification to a gene which modifies the expression, function, or activity of the gene can

have an impact on the action of other genes and their expression products within a given metabolic pathway (e.g., by inhibition or competition). In this embodiment, the action (e.g., activity) of a particular gene and/or its product can be affected (i.e., upregulated or downregulated) by a genetic modification to another gene within the same metabolic pathway, or to a gene within a different metabolic pathway which impacts the pathway of interest by competition, inhibition, substrate formation, etc.

In general, a plant or microorganism having a genetic modification that affects L-ascorbic acid production has at least one genetic modification, as discussed above, which results in a change in the L-ascorbic acid production pathway as compared to a wild-type plant or microorganism grown or cultured under the same conditions. Such a modification in an L-ascorbic acid production pathway changes the ability of the plant or microorganism to produce L-ascorbic acid. According to the present invention, a genetically modified plant or microorganism preferably has an enhanced ability to produce L-ascorbic acid compared to a wild-type plant or microorganism cultured under the same conditions.

The present invention is based on the present inventors' discovery of the biosynthetic pathway for L-ascorbic acid (vitamin C) in plants and microorganisms. Prior to the present invention, the metabolic pathway by which plants produce L-ascorbic acid, was not completely elucidated. The present inventors have demonstrated that L-ascorbic acid production in plants, including L-ascorbic acid-producing microorganisms (e.g., microalgae), is a pathway which uses GDP-D-mannose and involves sugar phosphates and NDP-sugars. In addition, the present inventors have made the surprising discovery that both L-galactose and L-galactono-γ-lactone can be rapidly converted into L-ascorbic acid in L-ascorbic acid-producing microalgae, including *Prototheca* and *Chlorella pyrenoidosa*. The entire pathway for L-ascorbic acid production in plants is set forth in Figs. 1A-1C. More particularly, Fig. 1A shows that the production of L-ascorbic acid in plants proceeds through the production of mannose intermediates to GDP-D-mannose, followed by the conversion of GDP-D-mannose to GDP-L-galactose by GDP-D-mannose:GDP-L-galactose epimerase (also known as GDP-D-mannose-3,5-epimerase) (Fig. 1B), and then by the subsequent progression to L-galactose-1-P, L-galactose, L-galactonic acid (optional), L-galactono-γ-lactone, and L-ascorbic acid (Fig. 1C). Fig. 1B

also illustrates alternate pathways for the use of various intermediates, such as GDP-D-mannose. Certain aspects of this pathway have been independently described in a publication (Wheeler, et al., 1998, *Nature* 393:365-369), incorporated herein by reference in its entirety.

Points within the L-ascorbic acid production pathway which can be targeted by genetic modification to affect the production of L-ascorbic acid can generally be catagorized into at least one of the following pathways: (a) pathways affecting the production of GDP-D-mannose (e.g., pathways for converting a carbon source into GDP-D-mannose); (b) pathways for converting GDP-D-mannose into other compounds, (c) pathways associated with or downstream of the action of GDP-D-mannose:GDP-L-galactose epimerase, (d) pathways which compete for substrates involved in the production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid; and (e) pathways which inhibit production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid.

A genetically modified plant or microorganism useful in a method of the present invention typically has at least one genetic modification in the L-ascorbic acid production pathway which results in an enhanced production of L-ascorbic acid. In one embodiment, a genetically modified plant or microorganism has at least one genetic modification that results in: (a) an enhanced production of GDP-D-mannose; (b) an inhibition of pathways which convert GDP-D-mannose into compounds other than GDP-L-galactose; (c) an enhancement of action of the GDP-D-mannose:GDP-L-galactose epimerase; (d) an enhancement of the action of enzymes downstream of the GDP-D-mannose:GDP-L-galactose epimerase; (e) an inhibition of pathways which compete for substrates involved in the production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid; and (e) an inhibition of pathways which inhibit production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-

galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid.

An enhanced production of GDP-D-mannose by genetic modification of the plant or microorganism can be achieved by, for example, overexpression of enzymes such as hexokinase, glucose phosphate isomerase, phosphomannose isomerase (PMI), phosphomannomutase (PMM) and/or GDP-D-mannose pyrophosphorylase (GMP). Inhibition of pathways which convert GDP-D-mannose to compounds other than GDP-L-galactose can be achieved, for example, by modifications which inhibit polysaccharide synthesis, GDP-D-rhamnose synthesis, GDP-L-fucose synthesis and/or GDP-D-mannuronic acid synthesis. An increase in the action of the GDP-D-mannose:GDP-L-galactose epimerase and of enzymes downstream of the epimerase in the L-ascorbic acid production pathway can be achieved by genetic modifications which include, but are not limited to: overexpression of the epimerase gene (i.e, by overexpression of a recombinant nucleic acid molecule encoding the epimerase gene or a homologue thereof (discussed in detail below), and/or by mutation of the endogenous or recombinant gene to enhance expression of the gene) and/or overexpression of genes downstream of the epimerase which encode subsequent enzymes in the L-ascorbic acid pathway. Finally, metabolic pathways which compete with or inhibit the L-ascorbic acid production pathway can be inhibited by deleting or mutating enzymes, substrates or products which either inhibit or compete for an enzyme, substrate or product in the L-ascorbic acid pathway.

As discussed above, a genetically modified plant or microorganism useful in the method of the present invention can have at least one genetic modification (e.g., mutation in the endogenous gene or addition of a recombinant gene) in a gene encoding an enzyme involved in the L-ascorbic acid production pathway. Such genetic modifications preferably increase (i.e., enhance) the action of such enzymes such that L-ascorbic acid is preferentially produced as compared to other possible end products in related metabolic pathways. Such genetic modifications include, but are not limited to, overexpression of the gene encoding such enzyme, and deletion, mutation, or downregulation of genes encoding competitors or inhibitors of such enzyme. Preferred enzymes for which the action of the gene encoding such enzyme can be genetically modified include: hexokinase, glucose phosphate isomerase, phosphomannose isomerase (PMI), phosphomannomutase

(PMM), GDP-D-mannose pyrophosphorylase (GMP), GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. More preferably, a genetically modified plant or microorganism useful in the present invention has a genetic modification which increases the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. Even more preferably, a genetically modified plant or microorganism useful in the present invention has a genetic modification which increases the action of GDP-D-mannose:GDP-L-galactose epimerase. These enzymes and the reactions catalyzed by such enzymes are illustrated in Figs. 1A-1C.

Prior to the present invention, without knowing the L-ascorbic acid biosynthetic (i.e., production) pathway, previous mutagenesis and screening efforts were limited in that only non-lethal mutations could be detected. One embodiment of the present invention relates to elimination of a key competing enzyme that diverts carbon flow from L-ascorbic acid synthesis. If such enzyme is absolutely required for growth on glucose, then mutants lacking the enzyme (and, therefore, having increased carbon flow to L-ascorbic acid) would have been nonviable and not have been detected during prior screening efforts. One such enzyme is phosphofructokinase (PFK) (See Fig. 2A). PFK is required for growth on glucose, and is the major step drawing carbon away from L-ascorbic acid biosynthesis (Fig. 2A). Elimination of PFK would render the cells nonviable on glucose-based media. Selection of a conditional mutant where PFK was inactivated by temperature shift, for example, may allow development of a L-ascorbic acid process where cell growth is achieved under permissive fermentation conditions, and L-ascorbic acid production (from glucose) is initiated by a shift to non-permissive condition. In this example, the temperature shift would eliminate carbon flow from glucose to glycolysis via PFK, thereby shunting carbon into the L-ascorbic acid branch of metabolism. This approach has application not only in natural L-ascorbic acid producing organisms, but also in L-ascorbic acid recombinant systems (genetically engineered plant or microorganisms) as discussed herein.

Knowing the identity and mechanism of the rate-limiting pathway enzymes in the L-ascorbic acid production pathway allows for design of specific inhibitors of the enzymes that are also growth inhibitory. Selection of mutants resistant to the inhibitors allows for the isolation of strains that contain L-ascorbic acid-pathway enzymes with more favorable kinetic properties. Therefore, one embodiment of the present invention is to identify inhibitors of the enzymes that are also growth inhibitory. These inhibitors are then used to select genetic mutants that overcome this inhibition and produce L-ascorbic acid at high levels. In this embodiment, the resultant plant or microorganism is a non-recombinant strain which can then be further modified by recombinant technology, if desired. In recombinant L-ascorbic acid producing strains, random mutagenesis and screening can be used as a final step to increase L-ascorbic acid production.

In yet another embodiment genetic modifications are made to an L-ascorbic acid producing organism directly. This allows one to build upon a base of data acquired during prior classical strain improvement efforts, and perhaps more importantly, allows one to take advantage of undefined beneficial mutations that occurred during classical strain improvement. Furthermore, fewer problems are encountered when expressing native, rather than heterologous, genes. The most advanced system for development of genetic systems for microalgae has been developed for *Chlamydomonas reinhardtii*. Preferably, development of such a genetically modified production organism would include: isolation of mutant(s) with a specific nutritional requirement for use with a cloned selectable marker gene (similar to the *ura3* mutants used in yeast and fungal systems); a cloned selectable marker such as *URA3* or alternatively, identification and cloning of a gene that specifies resistance to a toxic compound (this would be analogous to the use of antibiotic resistance genes in bacterial systems, and, as is the case in yeast and other fungi, a means of inserting/removing the marker gene repeatedly would be required, unless several different selectable markers were developed); a transformation system for introducing DNA into the production organism and achieving stable transformation and expression; and, a promoter system (preferably several) for high-level expression of cloned genes in the organism.

Another embodiment of the present invention, discussed in detail below, is to place key genes or allelic variants and homologues thereof from L-ascorbic acid producing

organisms (i.e., higher plants and microalgae) into a plant or microorganism that is more amenable to molecular genetic manipulation, including endogenous L-ascorbic acid producing microorganisms and suitable plants. For example, it is possible to identify a suitable non-pathogenic organism based on the requirement of growth (on glucose) at low pH (i.e., acid-tolerant organisms, discussed in detail below).

One suitable candidate for recombinant production in any suitable host organism is the gene (nucleic acid molecule) encoding GDP-D-mannose:GDP-L-galactose epimerase and homologues of the GDP-D-mannose:GDP-L-galactose epimerase, as well as any other epimerase that has structural homology at the primary (i.e., sequence) or tertiary (i.e., three dimensional) level, to a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase, or to a UDP-galactose 4-epimerase. Many microorganisms produce GDP-D-mannose as a precursor to exopolysaccharide and glycoprotein production, even though such organisms may not make L-ascorbic acid. This aspect of the present invention is discussed in detail below.

Referring to Figs. 1A-1C, at least some of the enzymes from glucose-6-phosphate to GDP-D-mannose are present in many organisms. In fact, the entire sequence is present in bacteria such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa*, and make up the early steps in the biosynthesis of the exopolysaccharide alginate. In this regard, it is possible that the only thing preventing these organisms from producing L-ascorbic acid could be the lack of GDP-D-mannose:GDP-L-galactose epimerase. The presence of PMI, PMM and GMP (see Fig. 1A) in so many organisms is important for two reasons. First, these organisms themselves could serve as alternate hosts for L-ascorbic acid production, by building on the existing early pathway enzymes and adding the required cloned genes (the epimerase and possibly others). Second, the genes encoding PMI, PMM and GMP can be cloned into a new organism where, together with the cloned epimerase, they would encode the overall pathway from glucose-6-phosphate to GDP-L- galactose.

In order to screen genomic DNA or cDNA libraries from different organisms and to isolate nucleic acid molecules encoding these enzymes such as the GDP-D-mannose:GDP-L-galactose epimerase, one can use any of a variety of standard molecular and biochemical techniques. For example, the GDP-D-mannose:GDP-L-galactose epimerase can be purified from an organism such as *Prototheca*, the N-terminal amino

acid sequence can be determined (including, if necessary, the sequence of internal peptide fragments), and this information can be used to design degenerate primers for amplifying a gene fragment from the organism's DNA. This fragment would then be used to probe the library, and subsequently fragments that hybridize to the probe would be cloned in that organism or another suitable production organism. There is ample precedent for plant enzymes being expressed in an active form in bacteria, such as *E. coli*. Alternatively, yeast are also a suitable candidate for developing a heterologous system for L-ascorbic acid production.

It is to be understood that the present invention discloses a method comprising the use of a microorganism with an ability to produce commercially useful amounts of L-ascorbic acid in a fermentation process (i.e., preferably an enhanced ability to produce L-ascorbic acid compared to a wild-type microorganism cultured under the same conditions). This method is achieved by the genetic modification of one or more genes encoding a protein involved in an L-ascorbic acid pathway which results in the production (expression) of a protein having an altered (e.g., increased or decreased) function as compared to the corresponding wild-type protein. Preferably, such genetic modification is achieved by recombinant technology. It will be appreciated by those of skill in the art that production of genetically modified plants or microorganisms having a particular altered function as described elsewhere herein (e.g., an enhanced ability to produce GDP-D-mannose:GDP-L-galactose epimerase), such as by transformation of the plant or microorganism with a nucleic acid molecule which encodes a particular enzyme, can produce many organisms meeting the given functional requirement, albeit by virtue of a variety of different genetic modifications. For example, different random nucleotide deletions and/or substitutions in a given nucleic acid sequence may all give rise to the same phenotypic result (e.g., decreased enzymatic activity of the protein encoded by the sequence). The present invention contemplates any such genetic modification which results in the production of a plant or microorganism having the characteristics set forth herein.

A microorganism to be used in the fermentation method of the present invention is preferably a bacterium, a fungus, or a microalga which has been genetically modified according to the disclosure above. More preferably, a microorganism useful in the present

invention is a microalga which is capable of producing L-ascorbic acid, although the present invention includes microorganisms which are genetically engineered to produce L-ascorbic acid using the knowledge of the key components of the pathway and the guidance provided herein. Even more preferably, a microorganism useful in the present invention is an acid-tolerant microorganism, such as microalgae of the genera *Prototheca* and *Chlorella*. Acid-tolerant yeast and bacteria are also known in the art. Acid-tolerant microorganisms are discussed in detail below. Particularly preferred microalgae include microalgae of the genera, *Prototheca* and *Chlorella*, with *Prototheca* being most preferred. All known species of *Prototheca* produce L-ascorbic acid. Production of ascorbic acid by microalgae of the genera *Prototheca* and *Chlorella* is described in detail in U.S. Patent No. 5,792,631, issued August 11, 1998, and in U.S. Patent No. 5,900,370, issued May 4, 1999, both of which are incorporated herein by reference in their entirety. Preferred bacteria for use in the present invention include, but are not limited to, *Azotobacter*, *Pseudomonas*, and *Escherichia*, although acid-tolerant bacteria are more preferred. Preferred fungi for use in the present invention include yeast, and more preferably, yeast of the genus, *Saccharomyces*. A microorganism for use in the fermentation method of the present invention can also be referred to as a production organism. According to the present invention, microalgae can be referred to herein either as microorganisms or as plants.

A preferred plant to genetically modify according to the present invention is preferably a plant suitable for consumption by animals, including humans. More preferably, such a plant is a plant that naturally produces L-ascorbic acid, although other plants can be genetically modified to produce L-ascorbic acid using the guidance provided herein.

The L-ascorbic acid production pathways of the microalgae *Prototheca* and *Chlorella pyrenoidosa* will be addressed as specific embodiments of the present invention are described below. It will be appreciated that other plants and, in particular, other microorganisms, have similar L-ascorbic acid pathways and genes and proteins having similar structure and function within such pathways. It will also be appreciated that plants and microorganisms which do not naturally produce L-ascorbic acid can be modified according to the present invention to produce L-ascorbic acid. As such, the principles

discussed below with regard to *Prototheca* and *Chlorella pyrenoidosa* are applicable to other plants and microorganisms, including genetically modified plants and microorganisms.

In one embodiment of the present invention, the action of an enzyme in the L-ascorbic acid production pathway is increased by amplification of the expression (i.e., overexpression) of an enzyme in the pathway, and particularly, the GDP-D-mannose:GDP-L-galactose epimerase, homologues of the epimerase, and/or enzymes downstream of the epimerase. Overexpression of an enzyme can be accomplished, for example, by introduction of a recombinant nucleic acid molecule encoding the enzyme. It is preferred that the gene encoding an enzyme in the L-ascorbic acid production pathway be cloned under control of an artificial promoter. The promoter can be any suitable promoter that will provide a level of enzyme expression required to maintain a sufficient level of L-ascorbic acid in the production organism. Preferred promoters are constitutive (rather than inducible) promoters, since the need for addition of expensive inducers is therefore obviated. The gene dosage (copy number) of a recombinant nucleic acid molecule according to the present invention can be varied according to the requirements for maximum product formation. In one embodiment, the recombinant nucleic acid molecule encoding a gene in the L-ascorbic acid production pathway is integrated into the chromosomes of the microorganism.

It is another embodiment of the present invention to provide a microorganism having one or more enzymes in the L-ascorbic acid production pathway with improved affinity for its substrates. An enzyme with improved affinity for its substrates can be produced by any suitable method of genetic modification or protein engineering. For example, computer-based protein engineering can be used to design an epimerase protein with greater stability and better affinity for its substrate. See for example, Maulik et al., 1997, *Molecular Biotechnology: Therapeutic Applications and Strategies*, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety.

Recombinant nucleic acid molecules encoding proteins in the L-ascorbic acid production pathway can be modified to enhance or reduce the function (i.e., activity) of the protein, as desired to increase L-ascorbic acid production, by any suitable method of genetic modification. For example, a recombinant nucleic acid molecule encoding an

enzyme can be modified by any method for inserting, deleting, and/or substituting nucleotides, such as by error-prone PCR. In this method, the gene is amplified under conditions that lead to a high frequency of misincorporation errors by the DNA polymerase used for the amplification. As a result, a high frequency of mutations are obtained in the PCR products. The resulting gene mutants can then be screened for enhanced substrate affinity, enhanced enzymatic activity, or reduced/increased inhibitory ability by testing the mutant genes for the ability to confer increased L-ascorbic acid production onto a test microorganism, as compared to a microorganism carrying the non-mutated recombinant nucleic acid molecule.

Another embodiment of the present invention includes a microorganism in which competitive side reactions are blocked, including all reactions for which GDP-D-mannose is a substrate other than the production of L-ascorbic acid. In a preferred embodiment, a microorganism having complete or partial inactivation (decrease in the action of) of genes encoding enzymes which compete with the GDP-D-mannose:GDP-L-galactose epimerase for the GDP-D-mannose substrate is provided. Such enzymes include GDP-D-mannase and/or GDP-D-mannose-dehydrogenase. As used herein, inactivation of a gene can refer to any modification of a gene which results in a decrease in the activity (i.e., expression or function) of such a gene, including attenuation of activity or complete deletion of activity.

As discussed above, a particularly preferred aspect of the method to produce L-ascorbic acid by fermentation of a genetically modified microorganism of the present invention includes the step of culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. According to the present invention, such an epimerase can include the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway, described above, as well as any other epimerase that has structural homology at the primary (i.e., sequence) or tertiary (i.e., three dimensional) level, to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or to a UDP-galactose 4-epimerase. Such structural homology is discussed in detail below. Preferably, such an epimerase is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. In one embodiment, the genetic modification includes transformation of the

microorganism with a recombinant nucleic acid molecule that expresses such an epimerase.

Therefore, the epimerase encompassed in the method and organisms of the present invention includes the endogenous epimerase which operates in the naturally occurring ascorbic acid biosynthetic pathway (referred to herein as GDP-D-mannose:GDP-L-galactose epimerase), GDP-4-keto-6-deoxy-D-mannose epimerase/ reductases, and any other epimerase which is capable of catalyzing the conversion of GDP-D mannose to GDP-L-galactose and which is structurally homologous to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase. An epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose according the present invention can be identified by biochemical and functional characteristics as well as structural characteristics. For example, an epimerase according to the present invention is capable of acting on GDP-D-mannose as a substrate, and more particularly, such an epimerase is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. It is to be understood that such capabilities need not necessarily be the normal or natural function of the epimerase as it acts in its endogenous (i.e., natural) environment. For example, GDP-4-keto-6-deoxy-D-mannose epimerase/reductase in its natural environment under normal conditions, catalyzes the conversion of GDP-D-mannose to GDP-L-fucose and does not act directly on GDP-D-mannose (See Fig.8A, B), however, such an epimerase is encompassed by the present invention for use in catalyzing the conversion of GDP-D-mannose to GDP-L-galactose for production of ascorbic acid, to the extent that it is capable of, or can be modified to be capable of, catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. Therefore, the present invention includes epimerases which have the desired enzyme activity for use in production of ascorbic acid, are capable of having such desired enzyme activity, and/or are capable of being modified or induced to have such desired enzyme activity.

In one embodiment, an epimerase according to the present invention includes an epimerase that catalyzes the reaction depicted in Fig. 7. In another embodiment, an epimerase according to the present invention includes an epimerase that catalyzes the first of the reactions depicted in Fig. 8B. In one embodiment, an epimerase according to the

present invention binds to NADPH. In another embodiment, an epimerase according to the present invention is NADPH-dependent for enzyme activity.

As discussed above, the present inventors have discovered that a key enzyme in L-ascorbic acid biosynthesis in plants and microorganisms is GDP-D-mannose:GDP-L-galactose epimerase (refer to Figs. 1A-1C). One embodiment of the invention described herein is directed to the manipulation of this enzyme and structural homologues of this enzyme to increase L-ascorbic acid production in genetically engineered plants and/or microorganisms. More particularly, the GDP-D-mannose:GDP-L-galactose epimerase of the L-ascorbic acid pathway and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases are believed to be structurally homologous at both the sequence and tertiary structure level; a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is believed to be capable of functioning in the L-ascorbic acid biosynthetic pathway; and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or homologue thereof may be superior to a GDP-D-mannose-GDP-L-galactose epimerase for increasing L-ascorbic acid production in genetically engineered plants and/or microorganisms. Furthermore, the present inventors disclose the use of a nucleotide sequence encoding all or part of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase as a probe to identify the gene encoding GDP-D-mannose:GDP-L-galactose epimerase. Similarly, the present inventors disclose the use of a nucleotide sequence of the gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase/reductase to design oligonucleotide primers for use in a PCR-based strategy for identifying and cloning a gene encoding GDP-D-mannose:GDP-L-galactose epimerase.

Without being bound by theory, the present inventors believe that the following evidence supports the novel concept that the GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases have significant structural homology at the level of sequence and/or tertiary structure, and that the GDP-4-keto-6-deoxy-D-mannose epimerase/reductases and/or homologues thereof would be useful for production of ascorbic acid and/or for isolating the endogenous GDP-D-mannose:GDP-L-galactose epimerase.

Although prior to the present invention, it was not known that the GDP-D-mannose:GDP-L-galactose epimerase enzyme (also known as GDP-D-mannose-3,5-epimerase) plays a critical role in L-ascorbic acid biosynthesis, this enzyme was previously

described to catalyze the overall reversible reaction between GDP-D-mannose and GDP-L-galactose (Barber, 1971, *Arch. Biochem. Biophys.* 147:619-623; Barber, 1975, *Arch. Biochem. Biophys.* 167:718-722; Barber, 1979, *J. Biol. Chem.* 254:7600-7603; Hebda, et al., 1979, *Arch. Biochem. Biophys.* 194:496-502; Barber and Hebda, 1982, *Meth. Enzymol.*, 83:522-525). Despite these studies, GDP-D-mannose:GDP-L-galactose epimerase has never been well characterized nor has the gene encoding this enzyme been cloned and sequenced. Since the original work by Barber, GDP-D-mannose:GDP-L-galactose epimerase activity has been detected in the colorless microalga *Prototheca moriformis* by the assignee of the present application, and in *Arabidopsis thaliana* and pea embryonic axes (Wheeler, et al., 1998, *ibid.*).

Barber (1979, *J. Biol. Chem.* 254:7600-7603) proposed a mechanism for GDP-D-mannose:GDP-L-galactose epimerase partially purified from the green microalga *Chlorella pyrenoidosa*. The overall conversion of GDP-D-mannose to GDP-L-galactose was proposed to proceed by oxidation of the hexosyl moiety at C-4 to a keto intermediate, ene-diol formation, and inversion of the configurations at C-3 and C-5 upon rehydration of the double bonds and stereospecific reduction of the keto group. The proposed mechanism is depicted in Fig. 7.

Based on Barber's work, Feingold and Avigad (1980, In *The Biochemistry of Plants*, Vol. 3: Carbohydrates; Structure and Function, P.K. Stompf and E.E. Conn, eds., Academic Press, NY) elaborated further on the proposed mechanism for GDP-D-mannose:GDP-L-galactose epimerase. This mechanism is based on the assumption that the epimerase contains tightly bound NAD^+, and transfer of a hydride ion from C-4 of the substrate (GDP-D-mannose) to enzyme-associated NAD^+ converts the enzyme to the reduced (NADH)form, generating enzyme-bound GDP-4-keto-D-mannose. The latter would then undergo epimerization by an ene-diol mechanism. The final product (GDP-L-galactose) would be released from the enzyme after stereospecific transfer of the hydride ion originally removed from C-4, simultaneously regenerating the oxidized form of the enzyme.

L-fucose (6-deoxy-L-galactose) is a component of bacterial lipopolysaccharides, mammalian and plant glycoproteins and polysaccharides of plant cell walls. L-fucose is synthesized *de novo* from GDP-D-mannose by the sequential action of GDP-D-mannose-

4,6-dehydratase (an NAD(P)-dependent enzyme), and a bifunctional GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (NADPH-dependent), also referred to in scientific literature as GDP-fucose synthetase (Rizzi, et al., 1998, *Structure* 6:1453-1465; Somers, et al., 1998, *Structure* 6:1601-1612). This pathway for L-fucose biosynthesis appears to be ubiquitous (Rizzi, et al., 1998, *Structure* 6:1453-1465). The mechanisms for GDP-D-mannose-4,6-dehydratase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase are shown in Fig. 8A, B (Chang, et al., 1988, *J. Biol. Chem.* 263:1693-1697; Barber, 1980, *Plant Physiol.* 66:326-329).

Comparison of Figs. 7 and 8A, B reveals that Barber's proposed mechanism for GDP-D-mannose:GDP-L-galactose epimerase is analogous to the reaction mechanism for GDP-4-keto-6-deoxy-D-mannose epimerase/reductase. The same mechanism has also been demonstrated for the epimerization reaction that occurs in the biosynthesis of two TDP-6-deoxy hexoses, TDP-L-rhamnose and TDP-6-deoxy-L-talose, from TDP-D-glucose (Liu and Thorson, 1994, *Ann. Rev. Microbiol.* 48:223-256). In the latter cases, however, the final reduction at C-4 is catalyzed by NADPH-dependent reductases that are separate from the epimerase enzyme. These reductases have opposite stereospecificity, providing either TDP-L-rhamnose or TDP-6-deoxy-L-talose (Liu and Thorson, 1994, *Ann. Rev. Microbiol.* 48:223-256).

In all of the mechanisms described above, NAD(P)H is required for the final reduction at C-4 (refer to Fig. 8B). In the work of Hebda, et al. (1979, *Arch. Biochem. Biophys.* 194:496-502), it was reported that GDP-D-mannose:GDP-L-galactose epimerase from *C. pyrenoidosa* did not require NAD, NADP or NADH for activity. Strangely, NADPH was not tested. Based on the analogous mechanisms shown in Figs. 7 and 8A, B, the present inventors believe that it is likely that GDP-D-mannose:GDP-L-galactose epimerase from *C. pyrenoidosa* requires NADPH for the final reduction step. Why activity was detected *in vitro* without NADPH addition is not known, but tight *binding of NADPH to the enzyme could explain this observation. On the other hand, if the proposed mechanism of Feingold and Avigad (1980, in *The Biochemistry of Plants*, Vol. 3, p. 101-170: Carbohydrates; Structure and Function, P.K. Stompf and E.E. Conn, ed., Academic Press, NY) is correct, the reduced enzyme-bound cofactor generated in the first oxidation step of the epimerase reaction would serve as the source of electrons for

the final reduction of the keto group at C-4 back to the alcohol. Thus no addition of exogenous reduced cofactor would be required for activity *in vitro*.

Recently, a human gene encoding the bifunctional GDP-4-keto-6-deoxy-D-mannose epimerase/reductase was cloned and sequenced (Tonetti, et al., 1996, *J. Biol. Chem.* 271-27274-27279). This amino acid sequence of the human GDP-4-keto-6-deoxy-D-mannose epimerase/reductase shows significant homology (29% identity) to the *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (Tonetti, et al., 1998, *Acta Cryst.* D54:684-686; Somers, et al., 1998, *Structure* 6:1601-1612, both of which are incorporated herein by reference in their entireties). Tonetti et al. and Somers et al. additionally disclosed the tertiary (three dimensional) structure of the *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (also known as GDP-fucose synthetase), and noted significant structural homology with another epimerase, UDP-galactose 4-epimerase (GalE). These epimerases also share significant homology at the sequence level. Since no gene encoding a GDP-D-mannose:GDP-L-galactose epimerase has been cloned and sequenced, homology with genes encoding GDP-4-keto-6-deoxy-D-mannose epimerase/reductases or with genes encoding a UDP-galactose 4-epimerase has not been demonstrated. However, based on the similarity of the reaction products for GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (i.e., GDP-L-galactose and GDP-6-deoxy-L-galactose [i.e., GDP-L-fucose], respectively) and the common catalytic mechanisms (Figs. 7 and 8A, B) the present inventors believe that the genes encoding the enzymes will have a high degree of sequence homology, as well as tertiary structural homology.

Significant structural homology between GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases may allow a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or a homologue thereof, to function in the L-ascorbic acid biosynthetic pathway, and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase could potentially be even better than a GDP-D-mannose-GDP-L-galactose epimerase for increasing L-ascorbic acid production in genetically engineered plants and/or microorganisms. Furthermore, a nucleotide sequence encoding all or part of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can be used as a probe to identify the gene encoding GDP-D-mannose:GDP-L-galactose epimerase. Likewise, the

nucleotide sequence of the gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase can be used to design oligonucleotide primers for use in a PCR-based strategy for identifying and cloning a gene encoding GDP-D-mannose:GDP-L-galactose epimerase.

The ability to substitute GDP-4-keto-6-D-mannose epimerase/reductase for GDP-D-mannose:GDP-L-galactose epimerase to enhance L-ascorbic acid biosynthesis in plants or microorganisms depends on the ability of GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase to act directly on GDP-D-mannose to form GDP-L-galactose. Evidence supporting this possibility already exists. *Arabidopsis thaliana murl* mutants are defective in GDP-D-mannose-4,6-dehydratase activity (Bonin, et al., 1997, *Proc. Natl. Acad. Sci.* 94:2085-2090). These mutants are thus blocked in GDP-L-fucose biosynthesis, and consequently have less than 2% of the normal amounts of L-fucose in the primary cell walls of aerial portions of the plant (Zablackis, et al., 1996, *Science* 272:1808-1810). The *murl* mutants are more brittle than wild-type plants, are slightly dwarfed and have an apparently normal life cycle (Zablackis, et al., 272:1808-1810). When *murl* mutants are grown in the presence of exogenous L-fucose, the L-fucose content in the plant is restored to the wild-type state (Bonin, et al., 1997, *Proc. Natl. Acad. Sci.* 94:2085-2090). It was discovered (Zablackis, et al., 1996, *Science* 272:1808-1810) that *murl* mutants contain, in the hemicellulose xyloglucan component of the primary cell wall, L-galactose in place of the normal L-fucose. L-galactose is not normally found in the xyloglucan component, but in *murl* mutants L-galactose partly replaces the terminal L-fucosyl residue. Bonin, et al. (1997, *Proc. Natl. Acad. Sci.* 94:2085-2090) hypothesized that in the absence of a functional GDP-D-mannose-4,6-dehydratase in the *murl* mutants, the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase normally involved in L-fucose synthesis may be able to use GDP-D-mannose directly, forming GDP-L-galactose. Another possibility, however, is that the enzymes involved in L-ascorbic acid biosynthesis in *A. thaliana* are responsible for forming GDP-L-galactose in the *murl* mutant. If this were true, it would suggest that in the wild-type plant, some mechanism exists that prevents GDP-L-galactose formed in the L-ascorbic acid pathway from entering cell wall biosynthesis and substituting for (competing with) GDP-L-fucose for incorporation into the xyloglucan

component (since L-galactose is not present in the primary cell wall of the wild-type plant).

Because of the similar reaction mechanisms of GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, and because of the evidence that GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can act directly on GDP-D-mannose to form GDP-L-galactose, the present inventors believe that genes encoding all epimerases and epimerase/reductases that act on GDP-D-mannose have high homology. As such, one aspect of the present invention relates to the use of any epimerase (and nucleic acid sequences encoding such epimerase) having significant homology (at the primary, secondary and/or tertiary structure level) to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or to a UDP-galactose 4-epimerase for the purpose of improving the biosynthetic production of L-ascorbic acid.

Therefore, as described above, one embodiment of the present invention relates to a method for producing ascorbic acid or esters thereof in a microorganism, which includes culturing a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. Also included in the present invention are genetically modified microorganisms and plants in which the genetic modification increases the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose.

According to the present invention, an increase in the action of the GDP-D-mannose:GDP-L-galactose epimerase in the L-ascorbic acid production pathway can be achieved by genetic modifications which include, but are not limited to overexpression of the GDP-D-mannose:GDP-L-galactose epimerase gene, a homologue of such gene, or of any recombinant nucleic acid sequence encoding an epimerase that is homologous in primary (nucleic acid or amino acid sequence) or tertiary (three dimensional protein) structure to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, such as by overexpression of a recombinant nucleic acid molecule encoding the epimerase gene or a homologue thereof, and/or by mutation of the endogenous or recombinant gene to enhance expression of the gene.

According to the present invention, an epimerase that has a tertiary structure that is homologous to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/

reductase is an epimerase that has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws (Table 12). In another embodiment, an epimerase that has a tertiary structure that is homologous to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase is an epimerase that has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS. As used herein, a "tertiary structure" or "three dimensional structure" of a protein, such terms being interchangeable, refers to the components and the manner of arrangement of the components in three dimensional space to constitute the protein. The use of the term "substantially conforms" refers to at least a portion of a tertiary structure of an epimerase which is sufficiently spatially similar to at least a portion of a specified three dimensional configuration of a particular set of atomic coordinates (e.g., those represented by Brookhaven Protein Data Bank Accession Code 1bws) to allow the tertiary structure of at least said portion of the epimerase to be modeled or calculated (i.e., by molecular replacement) using the particular set of atomic coordinates as a basis for estimating the atomic coordinates defining the three dimensional configuration of the epimerase.

More particularly, a tertiary structure that substantially conforms to a given set of atomic coordinates is a structure having an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, a structure that substantially conforms to a given set of atomic coordinates is a structure wherein such structure has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, such structure has the

recited average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, such structure has the recited average root-mean-square deviation (RMSD) value over about 100% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Methods to calculate RMSD values are well known in the art. Various software programs for determining the tertiary structural homology between one or more proteins are known in the art and are publicly available, such as QUANTA (Molecular Simulations Inc.).

A preferred epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose according to the method and genetically modified organisms of the present invention includes an epimerase that comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the tertiary structure of the substrate binding site of the epimerase has an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions

as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over about 100% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. The tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws is discussed in detail in Rizzi et al., 1998, *ibid.* Additionally, the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS is discussed in detail in Somers et al., 1998, *ibid.*

Another preferred epimerase according to the present invention includes an epimerase that comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the tertiary structure of the catalytic site of the epimerase has an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, the tertiary structure of the catalytic site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the catalytic site of the epimerase has the recited

average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the catalytic site of the epimerase has the recited average root-mean-square deviation (RMSD) value over 100% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In one embodiment, an epimerase encompassed by the present invention includes an epimerase that has a catalytic site which includes amino acid residues: serine, tyrosine and lysine. In a preferred embodiment, the tertiary structure positions of the amino acid residues serine, tyrosine and lysine substantially conform to the tertiary structure position of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws. The tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws is discussed in detail in Rizzi et al., 1998, *ibid.* Additionally, the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS is discussed in detail in Somers et al., 1998, *ibid.*

In an even more preferred embodiment, the above definition of "substantially conforms" can be further defined to include atoms of amino acid side chains. As used herein, the phrase "common amino acid side chains" refers to amino acid side chains that are common to both the structures which substantially conforms to a given set of atomic coordinates and the structure that is actually represented by such atomic coordinates. Preferably, a tertiary structure that substantially conforms to a given set of atomic coordinates is a structure having an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å over at least about 25% of the common amino acid side chains as

compared to the tertiary structure represented by the given set of atomic coordinates. In another embodiment, a structure that substantially conforms to a given set of atomic coordinates is a structure having the recited average root-mean-square deviation (RMSD) value over at least about 50% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates, and in another embodiment, such structure has the recited average root-mean-square deviation (RMSD) value over at least about 75% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates, and in another embodiment, such a structure has the recited average root-mean-square deviation (RMSD) value over 100% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates.

A tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can be modeled by a suitable modeling computer program such as MODELER (A. Sali and T.L. Blundell, *J. Mol. Biol.*, vol. 234:779-815, 1993 as implemented in the Insight II Homology software package (Insight II (97.0), MSI, San Diego)), using information, for example, derived from the following data: (1) the amino acid sequence of the epimerase; (2) the amino acid sequence of the related portion(s) of the protein represented by the specified set of atomic coordinates having a three dimensional configuration; and, (3) the atomic coordinates of the specified three dimensional configuration. Alternatively, a tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can be modeled using data generated from analysis of a crystallized structure of the epimerase. A tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can also be calculated by a method such as molecular replacement. Methods of molecular replacement are generally known by those of skill in the art (generally described in Brunger, *Meth. Enzym.*, vol. 276, pp. 558-580, 1997; Navaza and Saludjian, *Meth. Enzym.*, vol. 276, pp. 581-594, 1997; Tong and Rossmann, *Meth. Enzym.*, vol. 276, pp. 594-611, 1997; and Bentley, *Meth. Enzym.*, vol. 276, pp. 611-619, 1997, each of which are incorporated by this reference herein in their entirety) and are performed in a software program including, for example, XPLOR (Brunger, et al., *Science*, vol. 235, p. 458, 1987). In addition, a structure can be modeled using techniques generally described by,

for example, Sali, *Current Opinions in Biotechnology*, vol. 6, pp. 437-451, 1995, and algorithms can be implemented in program packages such as Homology 95.0 (in the program Insight II, available from Biosym/MSI, San Diego, CA). Use of Homology 95.0 requires an alignment of an amino acid sequence of a known structure having a known three dimensional structure with an amino acid sequence of a target structure to be modeled. The alignment can be a pairwise alignment or a multiple sequence alignment including other related sequences (for example, using the method generally described by Rost, *Meth. Enzymol.*, vol. 266, pp. 525-539, 1996) to improve accuracy. Structurally conserved regions can be identified by comparing related structural features, or by examining the degree of sequence homology between the known structure and the target structure. Certain coordinates for the target structure are assigned using known structures from the known structure. Coordinates for other regions of the target structure can be generated from fragments obtained from known structures such as those found in the Protein Data Bank maintained by Brookhaven National Laboratory, Upton, NY. Conformation of side chains of the target structure can be assigned with reference to what is sterically allowable and using a library of rotamers and their frequency of occurrence (as generally described in Ponder and Richards, *J. Mol. Biol.*, vol. 193, pp. 775-791, 1987). The resulting model of the target structure, can be refined by molecular mechanics (such as embodied in the program Discover, available from Biosym/MSI) to ensure that the model is chemically and conformationally reasonable.

According to the present invention, an epimerase that has a nucleic acid sequence that is homologous at the primary structure level (i.e., is a homologue of) to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase includes any epimerase encoded by a nucleic acid sequence that is at least about 15%, and preferably at least about 20%, and more preferably at least about 25%, and even more preferably, at least about 30% identical to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, and preferably to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. Similarly, an epimerase that has an amino acid sequence that is homologous to an amino acid sequence of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-

galactose 4-epimerase includes any epimerase having an amino acid sequence that is at least about 15%, and preferably at least about 20%, and more preferably at least about 25%, and even more preferably, at least about 30% identical to an amino acid sequence of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, and preferably to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10.

According to one embodiment of the present invention, homology or percent identity between two or more nucleic acid or amino acid sequences is performed using methods known in the art for aligning and/or calculating percentage identity. To compare the homology/percent identity between two or more sequences as set forth above, for example, a module contained within DNASTAR (DNASTAR, Inc., Madison, Wisconsin) can be used. In particular, to calculate the percent identity between two nucleic acid or amino acid sequences, the Lipman-Pearson method, provided by the MegAlign module within the DNASTAR program, is preferably used, with the following parameters, also referred to herein as the Lipman-Pearson standard default parameters:

(1) Ktuple = 2;

(2) Gap penalty = 4;

(3) Gap length penalty = 12.

Using the Lipman-Pearson method with these parameters, for example, the percent identity between the amino acid sequence for *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (SEQ ID NO:4) and human GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (FX) (SEQ ID NO:6) is 27.7%, which is comparable to the 27% identity described for these enzymes in Tonetti et al., 1998, *Acta Cryst.* D54:684-686.

According to another embodiment of the present invention, to align two or more nucleic acid or amino acid sequences, for example to generate a consensus sequence or evaluate the similarity at various positions between such sequences, a CLUSTAL alignment program (e.g., CLUSTAL, CLUSTAL V, CLUSTAL W), also available as a module within the DNASTAR program, can be used using the following parameters, also referred to herein as the CLUSTAL standard default parameters:

Multiple Alignment Parameters (i.e., for more than 2 sequences):

(1) Gap penalty = 10;

(2) Gap length penalty = 10;

Pairwise Alignment Parameters (i.e., for two sequences):

(1) Ktuple = 1;

(2) Gap penalty = 3;

(3) Window = 5;

(4) Diagonals saved = 5.

According to the present invention, a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase can be a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from any organism, including *Arabidopsis thaliana*, *Escherichia coli*, and human. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *Arabidopsis thaliana* is represented herein by SEQ ID NO:1. SEQ ID NO:1 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:2. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *Escherichia coli* is represented herein by SEQ ID NO:3. SEQ ID NO:3 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase having an amino acid sequence represented herein as SEQ ID NO:4. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *homo sapiens* is represented herein by SEQ ID NO:5. SEQ ID NO:5 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:6.

According to the present invention, a UDP-galactose 4-epimerase can be a UDP-galactose 4-epimerase from any organism, including *Escherichia coli* and human. A nucleic acid sequence encoding a UDP-galactose 4-epimerase from *Escherichia coli* is represented herein by SEQ ID NO:7. SEQ ID NO:7 encodes a UDP-galactose 4-epimerase having an amino acid sequence represented herein as SEQ ID NO:8. A nucleic acid sequence encoding a UDP-galactose 4-epimerase from *homo sapiens* is represented herein by SEQ ID NO:9. SEQ ID NO:9 encodes a UDP-galactose 4-epimerase having an amino acid sequence represented herein as SEQ ID NO:10.

In a preferred embodiment, an epimerase encompassed by the present invention has an amino acid sequence that aligns with the amino acid sequence of SEQ ID NO:11, for example using a CLUSTAL alignment program, wherein amino acid residues in the

amino acid sequence of the epimerase align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11, and preferably at least about 75% of non-Xaa residues in SEQ ID NO:11, and more preferably, at least about 90% of non-Xaa residues in SEQ ID NO:11, and even more preferably 100% of non-Xaa residues in SEQ ID NO:11. The percent identity of residues aligning with 100% identity with non-Xaa residues can be simply calculated by dividing the number of 100% identical matches at non-Xaa residues in SEQ ID NO:11 by the total number of non-Xaa residues in SEQ ID NO:11. A preferred nucleic acid sequence encoding an epimerase encompassed by the present invention include a nucleic acid sequence encoding an epimerase having an amino acid sequence with the above described identity to SEQ ID NO:11. Such an alignment using a CLUSTAL alignment program is based on the same parameters as previously disclosed herein. SEQ ID NO:11 represents a consensus amino acid sequence of an epimerase which was derived by aligning at least portions of amino acid sequences SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8, as described in Somers et al., 1998, *Structure* 6:1601-1612, and can be approximately duplicated using CLUSTAL.

In another embodiment, an epimerase encompassed by the present invention includes an epimerase that has a catalytic site which includes amino acid residues: serine, tyrosine and lysine. Preferably, such serine, tyrosine and lysine residues are located at positions in the epimerase amino acid sequence which align using a CLUSTAL alignment program with positions Ser105, Tyr134 and Lys138 of consensus sequence SEQ ID NO:11, with positions Ser109, Tyr138 and Lys142 of sequence SEQ ID NO:2, with positions Ser107, Tyr136 and Lys140 of SEQ ID NO:4, with positions Ser114, Tyr143 and Lys147 of sequence SEQ ID NO:6, with positions Ser124, Tyr149 and Lys153 of sequence SEQ ID NO:8 or with positions Ser132, Tyr157 and Lys161 of sequence SEQ ID NO:10.

In another embodiment, an epimerase that has an amino acid sequence that is homologous to an amino acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase includes any epimerase that has an amino acid motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly, which is found, for example in positions 8 through 14 of the consensus sequence SEQ ID NO:11, in positions 12 through 18 of SEQ ID NO:2, in positions 10 through 16 of SEQ ID NO:4, in positions 14 through 20 of SEQ ID NO:6, in positions

7 through 13 of SEQ ID NO:8, and in positions 9 through 15 of SEQ ID NO:10. Such a motif can be identified by its alignment with the same motif in the above-identified amino acid sequences using a CLUSTAL alignment program. Preferably, such motif is located within the first 25 N-terminal amino acids of the amino acid sequence of the epimerase.

In yet another embodiment, an epimerase encompassed by the present invention includes an epimerase that has a substrate binding site which includes amino acid residues that align using a CLUSTAL alignment program with at least 50% of amino acid positions Asn177, Ser178, Arg187, Arg209, Lys283, Asn165, Ser107, Ser108, Cys109, Asn133, Tyr136 and His179 of SEQ ID NO:4. Alignment with positions Ser107, Tyr136, Asn165, Arg209, is preferably with 100% identity (i.e., exact match of residue, under parameters for alignment).

In another embodiment of the present invention, an epimerase encompassed by the present invention comprises at least 4 contiguous amino acid residues having 100% identity with at least 4 contiguous amino acid residues of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters or by comparing an alignment using a CLUSTAL program with CLUSTAL standard default parameters. According to the present invention, the term "contiguous" means to be connected in an unbroken sequence. For a first sequence to have "100% identity" with a second sequence means that the first sequence exactly matches the second sequence with no gaps between nucleotides or amino acids.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that comprises at least 12 contiguous nucleic acid residues having 100% identity with at least 12 contiguous nucleic acid residues of a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters or by comparing an alignment using a CLUSTAL program with CLUSTAL standard default parameters.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that hybridizes under stringent

hybridization conditions to a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety (see specifically, pages 9.31-9.62). In addition, formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting varying degrees of mismatch of nucleotides are disclosed, for example, in Meinkoth et al., 1984, *Anal. Biochem.* 138, 267-284; Meinkoth et al., *ibid.*, is incorporated by reference herein in its entirety.

More particularly, stringent hybridization and washing conditions, as referred to herein, refer to conditions which permit isolation of nucleic acid molecules having at least about 70% nucleic acid sequence identity with the nucleic acid molecule being used to probe in the hybridization reaction, more particularly at least about 75%, and most particularly at least about 80%. Such conditions will vary, depending on whether DNA:RNA or DNA:DNA hybrids are being formed. Calculated melting temperatures for DNA:DNA hybrids are 10°C less than for DNA:RNA hybrids. In particular embodiments, stringent hybridization conditions for DNA:DNA hybrids include hybridization at an ionic strength of 6X SSC (0.9 M Na^+) at a temperature of between about 20°C and about 35°C, more preferably, between about 28°C and about 40°C, and even more preferably, between about 35°C and about 45°C. In particular embodiments, stringent hybridization conditions for DNA:RNA hybrids include hybridization at an ionic strength of 6X SSC (0.9 M Na^+) at a temperature of between about 30°C and about 45°C, more preferably, between about 38°C and about 50°C, and even more preferably, between about 45°C and about 55°C. These values are based on calculations of a melting temperature for molecules larger than about 100 nucleotides, 0% formamide and a G + C content of about 40%. Alternatively, T_m can be calculated empirically as set forth in Sambrook et al., *supra*, pages 9.31 to 9.62.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that comprises a nucleic acid

sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 or a fragment thereof, wherein the fragment encodes a protein that is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose, such as under physiological conditions. In another embodiment, an epimerase encompassed by the present invention comprises an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10 or a fragment thereof, wherein the fragment is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. It is to be understood that the nucleic acid sequence encoding the amino acid sequences identified herein can vary due to degeneracies. As used herein, nucleotide degeneracies refers to the phenomenon that one amino acid can be encoded by different nucleotide codons.

One embodiment of the present invention relates to a method to identify an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. Preferably, such a method is useful for identifying the GDP-D-mannose:GDP-L-galactose epimerase which catalyzes the conversion of GDP-D-mannose to GDP-L-galactose in the endogenous (i.e., naturally occurring L-ascorbic acid biosynthetic pathway of microorganisms and/or plants). Such a method can include the steps of: (a) contacting a source of nucleic acid molecules with an oligonucleotide at least about 12 nucleotides in length under stringent hybridization conditions, wherein the oligonucleotide is identified by its ability to hybridize under stringent hybridization conditions to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5; and, (b) identifying nucleic acid molecules from the source of nucleic acid molecules which hybridize under stringent hybridization conditions to the oligonucleotide. Nucleic acid molecules identified by this method can then be isolated from the source using standard molecular biology techniques. Preferably, the source of nucleic acid molecules is obtained from a microorganism or plant that has an ascorbic acid production pathway. Such a source of nucleic acid molecules can be any source of nucleic acid molecules which can be isolated from an organism and/or which can be screened by hybridization with an oligonucleotide such as a probe or a PCR primer. Such sources include genomic and cDNA libraries and isolated RNA.

In order to screen cDNA libraries from different organisms and to isolate nucleic acid molecules encoding enzymes such as the GDP-D-mannose:GDP-L-galactose epimerase and related epimerases, one can use any of a variety of standard molecular and biochemical techniques. For example, oligonucleotide primers, preferably degenerate primers, can be designed using the most conserved regions of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase nucleic acid sequence, and such primers can be used in a polymerase chain reaction (PCR) protocol to amplify the same or related epimerases, including the GDP-D-mannose:GDP-L-galactose epimerase from the ascorbic acid pathway, from nucleic acids (e.g., genomic or cDNA libraries) isolated from a desired organism (e.g., a microorganism or plant having an L-ascorbic acid pathway). Similarly, oligonucleotide probes can be designed using the most conserved regions of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase nucleic acid sequence and such probe can be used to identify and isolate nucleic acid molecules, such as from a genomic or cDNA library, that hybridize under conditions of low, moderate, or high stringency with the probe.

Alternatively, the GDP-D-mannose:GDP-L-galactose epimerase can be purified from an organism such as *Prototheca*, the N-terminal amino acid sequence can be determined (including the sequence of internal peptide fragments), and this information can be used to design degenerate primers for amplifying a gene fragment from the organism cDNA. This fragment would then be used to probe the cDNA library, and subsequently fragments that hybridize to the probe would be cloned in that organism or another suitable production organism. There is ample precedent for plant enzymes being expressed in an active form in bacteria, such as *E. coli*. Alternatively, yeast are also a suitable candidate for developing a heterologous system for L-ascorbic acid production.

As discussed above in general for increasing the action of an enzyme in the L-ascorbic acid pathway according to the present invention, in one embodiment of the present invention, the action of an epimerase that catalyzes the conversion of GDP-D-mannose to GDP-L-galactose is increased by amplification of the expression (i.e., overexpression) of such an epimerase. Overexpression of an epimerase can be accomplished, for example, by introduction of a recombinant nucleic acid molecule encoding the epimerase. It is preferred that the gene encoding an epimerase according to

the present invention be cloned under control of an artificial promoter. The promoter can be any suitable promoter that will provide a level of epimerase expression required to maintain a sufficient level of L-ascorbic acid in the production organism. Preferred promoters are constitutive (rather than inducible) promoters, since the need for addition of expensive inducers is therefore obviated. The gene dosage (copy number) of a recombinant nucleic acid molecule according to the present invention can be varied according to the requirements for maximum product formation. In one embodiment, the recombinant nucleic acid molecule encoding an epimerase according to the present invention is integrated into the chromosome of the microorganism.

It is another embodiment of the present invention to provide a microorganism having one or more epimerases according to the present invention with improved affinity for its substrate. An epimerase with improved affinity for its substrate can be produced by any suitable method of genetic modification or protein engineering. For example, computer-based protein engineering can be used to design an epimerase protein with greater stability and better affinity for its substrate. See for example, Maulik et al., 1997, *Molecular Biotechnology: Therapeutic Applications and Strategies*, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety.

As noted above, in the method for production of L-ascorbic acid of the present invention, a microorganism having a genetically modified L-ascorbic acid production pathway is cultured in a fermentation medium for production of L-ascorbic acid. An appropriate, or effective, fermentation medium refers to any medium in which a genetically modified microorganism of the present invention, when cultured, is capable of producing L-ascorbic acid. Such a medium is typically an aqueous medium comprising assimilable carbon, nitrogen and phosphate sources. Such a medium can also include appropriate salts, minerals, metals and other nutrients. One advantage of genetically modifying a microorganism as described herein is that although such genetic modifications can significantly alter the production of L-ascorbic acid, they can be designed such that they do not create any nutritional requirements for the production organism. Thus, a minimal-salts medium containing glucose as the sole carbon source can be used as the fermentation medium. The use of a minimal-salts-glucose medium for the L-ascorbic acid fermentation will also facilitate recovery and purification of the L-ascorbic acid product.

In one mode of operation of the present invention, the carbon source concentration, such as the glucose concentration, of the fermentation medium is monitored during fermentation. Glucose concentration of the fermentation medium can be monitored using known techniques, such as, for example, use of the glucose oxidase enzyme test or high pressure liquid chromatography, which can be used to monitor glucose concentration in the supernatant, e.g., a cell-free component of the fermentation medium. As stated previously, the carbon source concentration should be kept below the level at which cell growth inhibition occurs. Although such concentration may vary from organism to organism, for glucose as a carbon source, cell growth inhibition occurs at glucose concentrations greater than at about 60 g/L, and can be determined readily by trial. Accordingly, when glucose is used as a carbon source the glucose concentration in the fermentation medium is maintained in the range of from about 1 g/L to about 100 g/L, more preferably in the range of from about 2 g/L to about 50 g/L, and yet more preferably in the range of from about 5 g/L to about 20 g/L. Although the carbon source concentration can be maintained within desired levels by addition of, for example, a substantially pure glucose solution, it is preferred to maintain the carbon source concentration of the fermentation medium by addition of aliquots of the original fermentation medium. The use of aliquots of the original fermentation medium are desirable because the concentrations of other nutrients in the medium (e.g. the nitrogen and phosphate sources) can be maintained simultaneously. Likewise, the trace metals concentrations can be maintained in the fermentation medium by addition of aliquots of the trace metals solution.

In an embodiment of the fermentation process of the present invention, a fermentation medium is prepared as described above. This fermentation medium is inoculated with

an actively growing culture of genetically modified microorganisms of the present invention in an amount sufficient to produce, after a reasonable growth period, a high cell density. Typical inoculation cell densities are within the range of from about 0.1 g/L to about 15 g/L, preferably from about 0.5 g/L to about 10 g/L and more preferably from about 1 g/L to about 5 g/L, based on the dry weight of the cells. The cells are then grown to a cell density in the range of from about 10 g/L to about 100 g/L preferably from about

20 g/L to about 80 g/L, and more preferably from about 50 g/L to about 70 g/L. The residence times for the microorganisms to reach the desired cell densities during fermentation are typically less than about 200 hours, preferably less than about 120 hours, and more preferably less than about 96 hours.

The microorganisms useful in the method of the present invention can be cultured in conventional fermentation modes, which include, but are not limited to, batch, fed-batch, and continuous. It is preferred, however, that the fermentation be carried out in fed-batch mode. In such a case, during fermentation some of the components of the medium are depleted. It is possible to initiate fermentation with relatively high concentrations of such components so that growth is supported for a period of time before additions are required. The preferred ranges of these components are maintained throughout the fermentation by making additions as levels are depleted by fermentation. Levels of components in the fermentation medium can be monitored by, for example, sampling the fermentation medium periodically and assaying for concentrations. Alternatively, once a standard fermentation procedure is developed, additions can be made at timed intervals corresponding to known levels at particular times throughout the fermentation. As will be recognized by those in the art, the rate of consumption of nutrient increases during fermentation as the cell density of the medium increases. Moreover, to avoid introduction of foreign microorganisms into the fermentation medium, addition is performed using aseptic addition methods, as are known in the art. In addition, a small amount of anti-foaming agent may be added during the fermentation.

The present inventors have determined that high levels of magnesium in the fermentation medium inhibits the production of L-ascorbic acid due to repression of enzymes early in the production pathway, although enzymes late in the pathway (i.e., from L-galactose to L-ascorbic acid) are not negatively affected (See Examples). Therefore, in a preferred embodiment of the method of the present invention, the step of culturing is carried out in a fermentation medium that is magnesium (Mg^{2+}) limited. Even more preferably, the fermentation is magnesium limited during the cell growth phase. Preferably, the fermentation medium comprises less than about 0.5 g/L of Mg^{2+} during the cell growth phase of fermentation, and even more preferably, less than about 0.2 g/L of Mg^{2+}, and even more preferably, less than about 0.1 g/L of Mg^{2+}.

The temperature of the fermentation medium can be any temperature suitable for growth and ascorbic acid production, and may be modified according to the growth requirements of the production microorganism used. For example, prior to inoculation of the fermentation medium with an inoculum, the fermentation medium can be brought to and maintained at a temperature in the range of from about 20°C to about 45°C, preferably to a temperature in the range of from about 25°C to about 40°C, and more preferably in the range of from about 30°C to about 38°C.

It is a further embodiment of the present invention to supplement and/or control other components and parameters of the fermentation medium, as necessary to maintain and/or enhance the production of L-ascorbic acid by a production organism. For example, in one embodiment, the pH of the fermentation medium is monitored for fluctuations in pH. In the fermentation method of the present invention, the pH is preferably maintained at a pH of from about pH 6.0 to about pH 8.0, and more preferably, at about pH 7.0. In the method of the present invention, if the starting pH of the fermentation medium is pH 7.0, the pH of the fermentation medium is monitored for significant variations from pH 7.0, and is adjusted accordingly, for example, by the addition of sodium hydroxide. In a preferred embodiment of the present invention, genetically modified microorganisms useful for production of L-ascorbic acid include acid-tolerant microorganisms. Such microorganisms include, for example, microalgae of the genera *Prototheca* and *Chlorella* (See U.S. Patent No. 5,792,631, *ibid.* and U.S. Patent No. 5,900,370, *ibid.*).

The production of ascorbic acid by culturing acid-tolerant microorganisms provides significant advantages over known ascorbic acid production methods. One such advantage is that such organisms are acidophilic, allowing fermentation to be carried out under low pH conditions, with the fermentation medium pH typically less than about 6. Below this pH, extracellular ascorbic acid produced by the microorganism during fermentation is relatively stable because the rate of oxidation of ascorbic acid in the fermentation medium by oxygen is reduced. Accordingly, high productivity levels can be obtained for producing L-ascorbic acid with acid-tolerant microorganisms according to the methods of the present invention. In addition, control of the dissolved oxygen content to very low levels to avoid oxidation of ascorbic acid is unnecessary. Moreover, this

advantage allows for the use of continuous recovery methods because extracellular medium can be treated to recover the ascorbic acid product.

Thus, the present method can be conducted at low pH when acid-tolerant microorganisms are used as production organisms. The benefit of this process is that at low pH, extracellular ascorbic acid produced by the organism is degraded at a reduced rate than if the fermentation medium was at higher pH. For example, prior to inoculation of the fermentation medium with an inoculum, the pH of the fermentation medium can be adjusted, and further monitored during fermentation. Typically, the pH of the fermentation medium is brought to and maintained below about 6, preferably below 5.5, and more preferably below about 5. The pH of the fermentation medium can be controlled by the addition of ammonia to the fermentation medium. In such cases when ammonia is used to control pH, it also conveniently serves as a nitrogen source in the fermentation medium.

The fermentation medium can also be maintained to have a dissolved oxygen content during the course of fermentation to maintain cell growth and to maintain cell metabolism for L-ascorbic acid formation. The oxygen concentration of the fermentation medium can be monitored using known methods, such as through the use of an oxygen probe electrode. Oxygen can be added to the fermentation medium using methods known in the art, for example, through agitation and aeration of the medium by stirring or shaking. Preferably, the oxygen concentration in the fermentation medium is in the range of from about 20% to about 100% of the saturation value of oxygen in the medium based upon the solubility of oxygen in the fermentation medium at atmospheric pressure and at a temperature in the range of from about 30°C to about 40°C. Periodic drops in the oxygen concentration below this range may occur during fermentation, however, without adversely affecting the fermentation.

The genetically modified microorganisms of the present invention are engineered to produce significant quantities of extracellular L-ascorbic acid. Extracellular L-ascorbic acid can be recovered from the fermentation medium using conventional separation and purification techniques. For example, the fermentation medium can be filtered or centrifuged to remove microorganisms, cell debris and other particulate matter, and L-ascorbic acid can be recovered from the cell-free supernate by conventional methods, such

as, for example, ion exchange, chromatography, extraction, solvent extraction, membrane separation, electrodialysis, reverse osmosis, distillation, chemical derivatization and crystallization.

One such example of L-ascorbic acid recovery is provided in U.S. Patent No. 4,595,659 by Cayle, incorporated herein in its entirety be reference, which discloses the isolation of L-ascorbic acid from an aqueous fermentation medium by ion exchange resin adsorption and elution, which is followed by decoloration, evaporation and crystallization. Further, isolation of the structurally similar isoascorbic acid from fermentation medium by a continuous multi-bed extraction system of anion-exchange resins is described by K. Shimizu, *Agr. Biol. Chem.* 31:346-353 (1967), which is incorporated herein in its entirety by reference.

Intracellular L-ascorbic acid produced in accordance with the present invention can also be recovered and used in a variety of applications. For example, cells from the microorganisms can be lysed and the ascorbic acid which is released can be recovered by a variety of known techniques. Alternatively, intracellular ascorbic acid can be recovered by washing the cells to extract the ascorbic acid, such as through diafiltration.

Development of a microorganism with enhanced ability to produce L-ascorbic acid by genetic modification can be accomplished using both classical strain development and molecular genetic techniques, and particularly, recombinant technology (genetic engineering). In general, the strategy for creating a microorganism with enhanced L-ascorbic acid production is to (1) inactivate or delete at least one, and preferably more than one of the competing or inhibitory pathways in which production of L-ascorbic acid is negatively affected (e.g., inhibited), and more significantly to (2) amplify the L-ascorbic acid production pathway by increasing the action of a gene(s) encoding an enzyme(s) involved in the pathway.

In one embodiment, the strategy for creating a microorganism with enhanced L-ascorbic acid production is to amplify the L-ascorbic acid production pathway by increasing the action of GDP-D-mannose:GDP-L-galactose epimerase, as discussed above. Such strategy includes genetically modifying the endogenous GDP-D-mannose:GDP-L-galactose epimerase such that L-ascorbic acid production is increased, and/or expressing/overexpressing a recombinant epimerase that catalyzes the conversion

of GDP-D-mannose to GDP-L-galactose, which includes expression of recombinant GDP-D-mannose:GDP-L-galactose epimerase and/or homologues thereof, and of other recombinant epimerases such as GDP-4-keto-6-deoxy-D-mannose epimerase reductase and epimerases that share structural homology with such epimerase as discussed in detail above.

It is to be understood that a production organism can be genetically modified by recombinant technology in which a nucleic acid molecule encoding a protein involved in the L-ascorbic acid production pathway disclosed herein is transformed into a suitable host which is a different member of the plant kingdom from which the nucleic acid molecule was derived. For example, it is an embodiment of the present invention that a recombinant nucleic acid molecule encoding a GDP-D-mannose:GDP-L-galactose epimerase from a higher plant can be transformed into a microalgal host in order to overexpress the epimerase and enhance production of L-ascorbic acid in the microalgal production organism.

As previously discussed herein, in one embodiment, a genetically modified microorganism can be a microorganism in which nucleic acid molecules have been deleted, inserted or modified, such as by insertion, deletion, substitution, and/or inversion of nucleotides, in such a manner that such modifications provide the desired effect within the microorganism. A genetically modified microorganism is preferably modified by recombinant technology, such as by introduction of an isolated nucleic acid molecule into a microorganism. For example, a genetically modified microorganism can be transfected with a recombinant nucleic acid molecule encoding a protein of interest, such as a protein for which increased expression is desired. The transfected nucleic acid molecule can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transfected (i.e., recombinant) host cell in such a manner that its ability to be expressed is retained. Preferably, once a host cell of the present invention is transfected with a nucleic acid molecule, the nucleic acid molecule is integrated into the host cell genome. A significant advantage of integration is that the nucleic acid molecule is stably maintained in the cell. In a preferred embodiment, the integrated nucleic acid molecule is operatively linked to a transcription control sequence (described below) which can be induced to control expression of the nucleic acid molecule.

A nucleic acid molecule can be integrated into the genome of the host cell either by random or targeted integration. Such methods of integration are known in the art. For example, an *E. coli* strain ATCC 47002 contains mutations that confer upon it an inability to maintain plasmids which contain a ColE1 origin of replication. When such plasmids are transferred to this strain, selection for genetic markers contained on the plasmid results in integration of the plasmid into the chromosome. This strain can be transformed, for example, with plasmids containing the gene of interest and a selectable marker flanked by the 5'- and 3'-termini of the *E. coli lacZ* gene. The *lacZ* sequences target the incoming DNA to the *lacZ* gene contained in the chromosome. Integration at the *lacZ* locus replaces the intact *lacZ* gene, which encodes the enzyme β-galactosidase, with a partial *lacZ* gene interrupted by the gene of interest. Successful integrants can be selected for β-galactosidase negativity.

A genetically modified microorganism can also be produced by introducing nucleic acid molecules into a recipient cell genome by a method such as by using a transducing bacteriophage. The use of recombinant technology and transducing bacteriophage technology to produce several different genetically modified microorganism of the present invention is known in the art.

According to the present invention, a gene, for example the GDP-D-mannose:GDP-L-galactose epimerase gene, includes all nucleic acid sequences related to a natural epimerase gene such as regulatory regions that control production of the epimerase protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In another embodiment, a gene, for example the GDP-D-mannose:GDP-L-galactose epimerase gene, can be an allelic variant that includes a similar but not identical sequence to the nucleic acid sequence encoding a given GDP-D-mannose:GDP-L-galactose epimerase gene. An allelic variant of a GDP-D-mannose:GDP-L-galactose epimerase gene which has a given nucleic acid sequence is a gene that occurs at essentially the same locus (or loci) in the genome as the gene having the given nucleic acid sequence, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Allelic variants typically encode proteins having similar activity to that of the protein encoded by the gene to which they are being

compared. Allelic variants can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions). Allelic variants are well known to those skilled in the art and would be expected to be found within a given microorganism or plant and/or among a group of two or more microorganisms or plants.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation). As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (i.e., complete) gene or a portion thereof capable of forming a stable hybrid with that gene. An isolated nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications provide the desired effect within the microorganism. A structural homologue of a nucleic acid sequence has been described in detail above. Preferably, a homologue of a nucleic acid sequence encodes a protein which has an amino acid sequence that is sufficiently similar to the natural protein amino acid sequence that a nucleic acid sequence encoding the homologue is capable of hybridizing under stringent conditions to (i.e., with) a nucleic acid molecule encoding the natural protein (i.e., to the complement of the nucleic acid strand encoding the natural protein amino acid sequence). A nucleic acid molecule homologue encodes a protein homologue. As used herein, a homologue protein includes proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation,

amidation and/or addition of glycosylphosphatidyl inositol) in such a manner that such modifications provide the desired effect on the protein and/or within the microorganism (e.g., increased or decreased action of the protein).

A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *ibid.*). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, PCR amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid and/or by hybridization with a wild-type gene.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a gene involved in an L-ascorbic acid production pathway.

Knowing the nucleic acid sequences of certain nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules and/or (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions). Such nucleic acid molecules can be obtained in a variety of ways including traditional cloning techniques using oligonucleotide probes to screen appropriate libraries or DNA and PCR amplification of appropriate libraries or DNA using oligonucleotide primers. Preferred libraries to screen or from which to amplify nucleic acid molecule include bacterial and yeast genomic DNA libraries, and in particular, microalgal genomic DNA libraries. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

The present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host microorganism of the present invention. Such a vector can contain nucleic acid sequences that are not naturally found adjacent to the isolated nucleic acid molecules to be inserted into the vector. The vector can be either RNA or DNA and typically is a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of nucleic acid molecules. One type of recombinant vector, referred to herein as a recombinant molecule and described in more detail below, can be used in the expression of nucleic acid molecules. Preferred recombinant vectors are capable of replicating in a transformed bacterial cells, yeast cells, and in particular, in microalgal cells.

Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection and biolistics.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules operatively linked to an expression vector containing one or more transcription control sequences. The phrase, operatively linked, refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. In the present invention, expression vectors are typically plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in a yeast host cell, a bacterial host cell, and preferably a microalgal host cell.

Nucleic acid molecules of the present invention can be operatively linked to expression vectors containing regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression

of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in yeast or bacterial cells or preferably, in microalgal cells. A variety of such transcription control sequences are known to those skilled in the art.

It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into the host cell chromosome, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals, modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

The following experimental results are provided for the purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

The present example describes the elucidation of the pathway from glucose to L-ascorbic acid through GDP-D-mannose in plants.

Since the present inventors have previously shown that *Prototheca* makes L-ascorbic acid (AA) from glucose, it was worthwhile to examine cultures for some of the early conversion products of glucose. In the past, the present inventors had concentrated on pathways from glucose to organic acids, based on the published pathway of L-ascorbic acid synthesis in animals and proposed pathways in plants. The present inventors demonstrate herein that the pathway from glucose to L-ascorbic acid involves not organic acids, but rather sugar phosphates and nucleotide diphosphate sugars (NDP-sugars).

Prior to the present invention, it was known that all cells synthesize polysaccharides by first forming NDP-sugars. The sugar moiety is then incorporated into polymer, while the cleaved NDP is recycled. A variety of polysaccharides are known, and are usually named based on the relative proportions of the sugar residues in the polymers. For example, a "galactomannan" contains mostly galactose, and to a lesser degree, mannose residues. The "biopolymer" from *Prototheca* strains isolated by the present inventors was analyzed and found to be 80% D-galactose, 18% rhamnose (D- or L-configuration not determined), and 2% L-arabinose. The present inventors provide evidence herein of how the respective NDP-sugars that make up the *Prototheca* biopolymer are formed, and what correlations exist between L-ascorbic acid synthesis and the formation of the NDP-sugar forms of the sugar residues found in the biopolymer.

The common NDP-sugar UDP-glucose is shown in Fig. 2B. This is formed in plants from glucose-l-P by the action of UDP-D-glucose pyrophosphorylase. UDP-glucose can be epimerized in plants to form UDP-D-galactose, using UDP-D-glucose-4-epimerase. UDP-D-galactose can also be formed by phosphorylation of D-galactose by galactokinase to form D-galactose-l-P, which can be converted to UDP-D-galactose by UDP-D-galactose pyrophosphorylase. These known routes were believed to account for the D-galactose in the *Prototheca* biopolymer. The UDP-L-arabinose can be formed by known reactions beginning with the oxidation of UDP-D-glucose to UDP-D-glucuronic acid (by UDP-D-glucose dehydrogenase), decarboxylation to UDP-D-xylose, and epimerization to UDP-L-arabinose. This accounts for the arabinose residues in the

biopolymer. UDP-L-rhamnose is known to be formed from UDP-D-glucose, thus all three of the sugar moieties in the *Prototheca* biopolymer can be accounted for by a pathway through glucose-1-P and UDP-glucose. Alternatively, if the rhamnose in the biopolymer is D-rhamnose, it is not formed via UDP-D-glucose, but by oxidation of GDP-D-mannose (See Fig. 1).

GDP-D-rhamnose is formed by converting glucose, in turn, to D-glucose-6-P, D-fructose-6-P, D-mannose-6-P, D-mannose-1-P, GDP-D-mannose, and GDP-D-rhamnose. It was of interest to the present inventors that this route passes through GDP-D-mannose. Exogenous mannose is known to be converted to D-mannose-6-P in plants, and can enter the path above. D-mannose is converted to L-ascorbic acid by *Prototheca* cells cultured by the present inventors as well or better than glucose (see Example 4). The mechanism of conversion, in *Chlorella pyrenoidosa* , of GDP-D-mannose to GDP-L-galactose by GDP-D-mannose:GDP-L-galactose epimerase, has been known for years (See, Barber, 1971, *Arch. Biochem. Biophys.* 147:619-623, incorporated herein by reference in its entirety). The present inventors have discovered herein that L-galactose and L-galactono-γ-lactone are rapidly converted to L-ascorbic acid by strains *of Prototheca* and *Chlorella pyrenoidosa*. Prior to the present invention, it was known that L-galactono-γ-lactone is converted to L-ascorbic acid in several plant systems, but the synthesis steps prior to this step were unknown. Based on the published literature and the present experimental evidence, the present inventors have determined that the L-ascorbic acid biosynthetic pathway in plants passes through GDP-D-mannose and involves sugar phosphates and NDP-sugars. The proposed pathway is shown in Fig. 1. Salient points relevant to the design and production of genetically modified microorganisms useful in the present method include:

1. The enzymes leading from D-glucose to D-fructose-6-P are well known enzymes in the first, uncommitted steps of glycolysis.

2. The enzymes involved in the conversion of D-fructose-6-P to GDP-D-mannose have been well characterized in plants, yeast, and bacteria, particularly *Azotobacter vinelandii and Pseudomonas aeruginosa,* which convert GDP-D-mannose to GDP-D-mannuronic acid, which is the precursor for alginate (See for example, Sa-Correia et al., 1987, *J. Bacteriol.* 169:3224-3231; Koplin et al., 1992, *J. Bacteriol.* 174:191-199; Oesterhelt et al., 1996, *Plant Science* 121:19-27; Feingold et al., 1980, The

Biochemistry of Plants: Vol 3: Carbohydrates, structure and function, P.K. Stampf & E.E. Conn, eds., Academic Press, New York, pp. 101-170; Smith et al., 1992, *Mol. Cell Biol.* 12:2924-2930; Boles et al., 1994, *Eur. J. Med.* 220:83-96; Hashimoto et al., 1997, *J. Biol. Chem.* 272:16308-16314, all of which are incorporated herein by reference in their entirety).

3. Barber (1971, *supra*, and 1975) identified in *Chlorella pyrenoidosa* the enzyme activities for the conversion of GDP-D-mannose to GDP-L-galactose and L-galactose-l-P.

4. The present inventors have shown herein the rapid conversion of L-galactose and L-galactono-γ-lactone to L-ascorbic acid by *Prototheca* cells.

5. L-galactono-γ-lactone and L-galactonic acid can be interconverted in solution by changing the pH of the solution; addition of base shifts the equilibrium to L-galactonic acid, while addition of acid shifts the equilibrium to the lactone. Cells may have an enzymatic means for this conversion in addition to this non-enzymatic route.

6. In plants, GDP-L-fucose is also formed from GDP-D-mannose, presumably for incorporation into polysaccharide. Roberts (1971) fed labeled D-mannose to corn root tips and found the label in polysaccharide, specifically in the residues of D-mannose, L-galactose, and L-fucose. No label was detected in D-glucose, D-galactose, L-arabinose, or D-xylose. *Prototheca and C. pyrenoidosa* cells have the ability to convert L-fucose (6-deoxy-L-galactose) to a dipyridyl-positive product that was shown by HPLC not to be L-ascorbic acid. The present inventors believe that it is was the 6-deoxy analog of L-ascorbic acid.

Example 2

This example shows that in *Prototheca*, like other plants (Loewus, F.A. 1988. In: J. Priess (ed.), The Biochemistry of Plants, 14:85-107. New York, Academic Press) and the green microalga *Chlorella pyrenoidosa* (Renstrom, *et al.*, 1983. Plant Sci. Lett. 28:299-305), ascorbic acid (AA) production from glucose proceeds by a biosynthetic pathway that allows retention of the configuration of the carbon skeleton of glucose.

Cultures of the strain UV77-247 were grown to moderate cell density in shake flasks with 1-^{13}C-labeled glucose as 10% of the total glucose (40 g/L). Incubation was

as per the standard Mg-limited screen (see Example 3). The culture supernates were clarified, deionized to remove salts, lyophilized, and subjected to nuclear magnetic resonance (nmr) analysis to determine where in the AA molecule the ^{13}C was located. In each case, approximately 85% of the label was found at the C-1 position of AA, with most of the remaining label at the C-6 position. This strongly indicated that AA is synthesized from glucose by a pathway that retains the carbon chain configuration, *i. e.*, C-1 of glucose becomes C-1 of AA. This has typically been observed in plants (Loewus, F.A. 1988. Ascorbic acid and its metabolic products. In: The Biochemistry of Plants, ed. J. Priess, 14:85-107. New York, Academic Press). Animals (Mapson, L.W. and F.A. Isherwood 1956. Biochem. J. 64:151-157; Loewus, F.A. 1960. J. Biol. Chem. 235(4):937-939) and protists such as *Euglena* (Shigeoka, S., *et al.*, 1979. J. Nutr. Sci. Vitaminol. 25:299-307), on the other hand, synthesize AA by a pathway that involves the inversion of configuration, *i. e.*, C-1 of glucose becomes C-6 of AA. Demonstration of the inversion/non-inversion nature of the pathway was an important step in determining the pathway of AA biosynthesis since the two types of pathways require different types of enzymatic reactions. The label found at C-6 of AA is thought to be due to metabolism of glucose and subsequent gluconeogenesis. The metabolism of glucose in glycolysis proceeds through triose phosphate intermediates. After this, the C-1 and C-6 carbons of glucose become biochemically equivalent. Hexose phosphates can be regenerated from the triose phosphates by gluconeogenesis, which is essentially a reversal of the degradative pathway. Consequently, metabolism of C-1-labeled glucose to triose phosphates with subsequent gluconeogenesis would result in the formation of hexose phosphate molecules labeled at either or both C-1 and C-6. If those hexose phosphates were precursors to AA, one would expect the AA to be similarly labeled. Consistent with this type of "isotopic mixing" is the observation that sucrose obtained from 1-^{13}C-labeled glucose was labeled at positions 1, 6, 1' and 6'.

Glucose can also be metabolized by the pentose phosphate pathway, the overall balanced equation for which is:

3 Glucose-6-phosphate → 2 Fructose-6-phosphate + Glyceraldehyde-3-phosphate + 3 CO_2

Based on the known biochemistry, it would then be expected that the label at each of the carbons in glucose (Table 1 left column) would appear at the positions for the other molecules shown, and that these patterns would be reflected in the AA formed from C-2- and C-3-labeled glucose.

TABLE 1

Predicted Carbon Labeling of Metabolites of Glucose in the Pentose Phosphate Pathway

Labeled Glucose Carbon	Position of Labeled Carbon in:			
	CO_2	F6P(1)	F6P(2)	G3P
1	+	-	-	-
2	-	1,3	1	-
3	-	2	2,3	-
4	-	4	4	1
5	-	5	5	2
6	-	6	6	3

AA recovered from cultures fed glucose labeled at C-2 or C-3 was also analyzed for its labeling patterns (Table 2).

TABLE 2

Labeling Pattern in AA after Cells were Fed 2-^{13}C and 3-^{13}C-glucose

Carbon Position in AA	Isotopic enhancement after growth on:	
	C-2 labeled glucose	C-3 labeled glucose
1	1.0	0.4
2	10.0	0.9
3	0.5	9.9
4	0	2.8
5	2.2	0.2
6	0	0

The data above again suggest a pathway from glucose to AA that proceeds by retention of configuration. As in the experiments with C-1 labeled glucose, approximately one-fifth of the label is present in "mirror image" position to the glucose label (C-5 for C-2 labeled glucose and C-4 for C-3 labeled glucose), indicating levels of gluconeogenesis consistent with those previously observed.

The small, but significant amount of enhancement observed in other positions is consistent with flux through the pentose phosphate pathway. As predicted above, carbon

flux through this pathway would result in isotopic enhancement at positions 1 and 3 when cells were grown on 2-^{13}C glucose and enhancement at position 2 when cells were grown on 3-^{13}C glucose. This is indeed observed. That there is twice as much enhancement at C-1 as there is at C-3 after growth on 2-^{13}C glucose is also predicted. These data indicate a small but measurable amount of carbon flux through the pentose phosphate pathway.

Example 3

This example shows the methods for generating, screening and isolating mutants of *Prototheca* with altered AA productivities compared to the starting strain ATCC 75669.

ATCC No. 75669, identified as *Prototheca moriformis* RSP1385 (unicellular green microalga), was deposited on February 8, 1994, with the American Type Culture Collection (ATCC), Rockville, Maryland, 20852, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Initial screening of *Prototheca* species and strains was reported in U.S. Patent No. 5,900,370, *ibid.* Table 3 lists the formulations of the media for growth and maintenance of the strains. Glucose for fermentors was supplied as glucose monohydrate and calculated on an anhydrous basis. The recipe for the trace metals solution is given in Table 4. The standard growth temperature was 35°C. All organisms were cultured axenically.

TABLE 3
Media for Growth and Maintenance of *Prototheca* Strains
All quantities are in g/L unless otherwise specified

Ingredient	Liquid		Agar		
	Standard	Mg-limiting	Slants	Ferrozine Plates	Standard Plates
Potassium phosphate monobasic	1.3	1.3	2.0	0.27	2.0
Potassium phosphate dibasic	3.8	3.8	2.0	1.4	2.0
Trisodium citrate dihydrate	7.7	7.7	2.6	1.3	2.6
Magnesium sulfate heptahydrate	0.40	0.02	0.4	0.01	0.4
Ammonium sulfate	3.7	3.7	1.0	1.0	1.0
Trace Metals Solution	2 mL	2 mL	2 mL	2 mL	2 mL
Ferrous sulfate heptahydrate	1.5 mg	4.5 mg	1.5 mg	-	1.5 mg
Calcium chlorid dihydrate	-	0.25	-	-	-

Ingredient	Liquid		Agar		
	Standard	Mg-limiting	Slants	Ferrozine Plat s	Standard Plates
Mangan us sulfate monohydrate	-	0.08	-	-	-
Yeast extract	-	-	2.5	-	-
Agar	-	-	15	15 (Noble)	15
pH before autoclaving	7.2	7.2	7.2	7.2	7.2
Autoclave, then add					
Copper sulfate, pentahydrate, 100 g/L	-	-	-	2 mL	-
40 g/L Ferrozine in 5 mM phosphate (pH 7.5 final)	-	-	-	8.8 mL	-
Ferric ammonium sulfate dodecahydrate, 40 g/L	-	-	-	3.8 mL	-
50% glucose with 25 mg/L thiamine HCl	40 mL	60 mL	10 mL	10 mL	10 mL

TABLE 4

Trace Metals Solution

Compound	Molecular Weight	Conc. of Individ. Solutions, g/L	mL Indiv. Stock per liter of Working Stock
Distilled Water	—	—	823
Hydrochloric Acid	—	Conc.	20
Cobalt Chloride hexahydrate	237.9	24.0	6.5
Boric acid	61.8	38.1	24
Zinc sulfate heptahydrate	287.5	35.3	50
Manganous sulfate monohydrate	169.0	24.6	50
Sodium molybdate dihydrate	242.0	23.8	2.0
Calcium chloride dihydrate	147.0	—	11.4 g
Vanadyl sulfate dihydrate	199.0	10.0	8.0
Nickel nitrate hexahydrate	290.8	5.0	8.0
Sodium selenite	173.0	5.0	8.0

Mutant isolates were generated by treatment with one or more of the following agents: nitrous acid (NA); ethyl methane sulfonate (EMS); or ultraviolet light (UV). Typically, glucose-depleted cells grown in standard liquid medium were washed and resuspended in 25 mM phosphate buffer, pH 7.2, diluted to approximately 10^7 colony-forming units per mL (cfu/mL), exposed to the mutagen to achieve about 99% kill, incubated 4-8 hours in the dark, and spread onto standard agar medium, or agar media containing differential agents.

Some mutant colonies on standard agar medium were picked randomly and subcultured to master plates. Other isolation plates were inverted over chloroform to lyse cells on the surface of the colonies and allow them to release AA. Released AA was detected by spraying the treated plates with a solution of 2,6-dichrorophenol-indophenol (1.25 g/L in 70% EtOH). The ability of AA to reduce this blue redox dye to its colorless form is the basis for a standard assay of AA (Omaye, *et al.*, 1979. Meth. Enzymol. 62:3-11.). Colonies derived from mutagenized cells were saved to master plates for further evaluation if their clear halos were significantly larger than the halos typical of the other mutants in that group. Other mutagenized cells were spread onto plates containing an AA detection system incorporated directly into the agar. This system is based on the ability of AA to reduce ferric iron to ferrous iron. The compound ferrozine (3-(2-pyridyl)-5,6- bis(4-phenylsulfonic acid)-1,2,4-triazine) was present in the agar to complex with the ferrous iron and give a violet color reaction. The ferrozine agar formulation is shown in Table 3. Colonies giving the darkest color reactions were master-plated. When screening for non-AA-producing strains (blocked mutants), white colonies were chosen against a background of relatively dark colonies.

For primary screening of tube cultures, cells were inoculated from master plates into 4 mL of Mg-limiting medium in 16 x 125 mm test tubes, and tubes were shaken in a slanted position on a rotary shaker at 300 rpm for four days. After both three and four days of incubation aliquots were removed for AA assay and cell density determination. Those for AA assay were centrifuged at 1500 x g for 5 min and the resulting supernates were removed for either colorimetric assay or high pressure liquid chromatography (HPLC). Promising isolates were retested in tube culture. Those passing the tube screen were tested in shake flasks.

For secondary screening of flask cultures, cells were inoculated into 50 mL of standard flask medium in 250 mL baffled shake flasks, and incubated on a rotary shaker at 180 rpm until glucose depletion (24-48 hours). A second series of flasks of Mg-sufficient standard medium was inoculated from the first set to a cell density of 0.15 A_{620}, and incubated for 24 hours. A third series of Mg-limiting flask medium was inoculated from the second set by a 1/50 dilution and incubated for 96 hours. Flasks were sampled for AA analysis and cell density measurements during this time as required.

Aliquots for supernatant AA analysis were centrifuged at 5000 x g for 5 min. Aliquots for total whole broth AA analysis were first extracted for 15 min with an equal volume of 5% trichloroacetic acid (TCA) before centrifugation. Aliquots of the resulting supernates were removed for either colorimetric assay or HPLC analysis.

For colorimetric assay of AA, a modification of the method of Omaye, *et al.* (1979. Meth. Enzymol. 62:3-11) was used. Twenty-five µL aliquots of culture supernates were added to wells of 96-well microplates, and 125 µL of color reagent was added. The color reagent consisted of four parts 0.5% aqueous 2,2'-dipyridyl and one part 8.3 mM ferric ammonium sulfate in 27 % (v/v) *o*-phosphoric acid, the two components being mixed immediately before use. After one hour, the absorbance at 520 nm was read. AA concentration was calculated by comparison of the absorbances of AA standards.

HPLC analysis was based on that of Running, *et al.*, (1994). Supernates were chromatographed on a Bio-Rad HPX-87H organic acid column (Bio-Rad Laboratories, Richmond, CA) with 13 mM nitric acid as solvent, at a flow rate of 0.7 mL/min at room temperature. Detection was at either 254 nm using a Waters 441 detector (Millipore Corp., Milford, MA), or at 245 nm using a Waters 481 detector. This system can distinguish between the L- and D- isomers of AA.

For dry weight determinations of cell density, 5 mL whole broth samples were centrifuged at 5000 x g for 5 min, washed once with distilled water, and the pellet was washed into a tared aluminum weighing pan. Cells were dried for 8-24 h at 105°C. Cell weight was calculated by difference.

Table 5 shows the abilities of various mutants of *Prototheca* to synthesize AA.

TABLE 5

AA Synthesizing Ability of Various *Prototheca* Mutants in Flask Screen

Strain	Specific AA Formation, mg AA per L/Culture A_{620}, during Mg-limited Incubation	
	2 Days Incubation	4 Days Incubation
ATCC 75669	22	35
EMS13-4	79	166
UV213-1	0	0
UV218-1	0	0
UV244-1	0	0

Strain	Specific AA Formation, mg AA per L/Culture A_{620}, during Mg-limited Incubation	
	2 Days Incubati n	4 Days Incubation
UV244-15	58	68
UV77-247	56	83
UV140-1	67	100
UV164-6	91	131
NA21-14	27	78
UV82-21	0	0
UV127-10	50	95
SP2-3	3	4

The genealogy of these isolates is presented graphically in the "family tree" of Fig. 3. ATCC No. _____, identified as *Prototheca moriformis* EMS13-4 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. ATCC No. _____, identified as *Prototheca moriformis* UV127-10 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. ATCC No. _____, identified as *Prototheca moriformis* SP2-3 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure.

Example 4

The following example shows that both growing and resting cells of *Prototheca* can rapidly convert L-galactose and L-galactono-γ-lactone to AA, and that conversion of D-mannose to AA by *Prototheca* is more rapid than conversion of D-glucose.

Shake flask cultures of the mutant strain UV77-247 were grown to glucose depletion in standard liquid medium (Table 3). Cells were washed twice and resuspended in complete medium with the glucose substituted by one of the compounds listed below.

Cell suspensions were incubated for 24 hours at 35° C with shaking, and the entire suspension was extracted with TCA as above and assayed for AA.

Tables 6-8 show that both growing and resting cells of strain UV77-247 can rapidly convert L-galactose and L-galactono-γ-lactone to AA. In these experiments, D-fructose and D-galactose were converted to AA at the same rate as D-glucose, suggesting that they are metabolized to AA through the same route as D-glucose. None of the organic acids suggested in the literature to be intermediates in the biosynthesis of AA were converted to AA, including sorbosone, which has been proposed as an intermediate by Saito *et al.*(1990 Plant Physiol. 94:1496-1500).

TABLE 6

Conversion of Compounds by Resting Cells of Strain UV77-247

Substrate (50 mM)	Total AA, mg/L	AA Relative to No Substrate Control
L-galactose	965	623
L-galactono-γ-lactone	818	476
D-fructose	590	248
D-glucosone	589	247
D-glucose	584	242
D-galactose	542	200
D-glucose (10 mM)	388	46
D-gluconolactone	382	40
D-gulono-γ-lactone	366	24
D-glucuronate	364	22
L-sorbosone	342	0
None	342	0
2-keto-D-gluconic acid	341	-1
D-isoascorbic acid (10 mM)	330	-12
D-glucuronolactone	329	-13
D-gluconic acid	309	-33
D-galacturonic acid	297	-45
L-idonate	296	-46

Since strain UV77-247 converted L-galactose and L-galactono-γ-lactone to AA much more rapidly than it did glucose, it suggests that these compounds are intermediates in the AA biosynthetic pathway and that they are "downstream" from glucose.

The data in Tables 7 and 8 also show that growing and resting cells of UV77-247 consistently convert D-mannose to AA at a rate greater than that of glucose.

TABLE 7

Conversion of Compounds t AA by Resting Cells of Strain UV77-247

Compound	Ascorbic Acid, mg/L		
	25.5 h	30 h	47 h
L-galactose	667	718	620
L-galactono-γ-lactone	644	681	749
D-glucosone	465	462	354
D-mannose	448	462	399
D-fructose	402	408	367
d-glucose	395	404	351
D-galactose	352	361	337
none	287	288	258

TABLE 8

Conversion of Compounds to AA by Growing Cells of Strain UV77-247

Compound	Ascorbic Acid, mg/L		A_{620}	AA/A_{620}
	25.5 h	44 h		
L-galactose	249	506	4.5	112
D-mannose	228	488	5.6	87
L-galactono-γ-lactone	214	342	5.0	68
D-glucose	178	398	5.9	67
D-fructose	181	383	5.9	65
D-glucosone	176	362	5.7	64
D-galactose	185	380	5.9	64
none	182	249	4.4	57
D-gluconic acid (K)	178	262	5.0	52
L-idonate (Na)	182	232	4.7	49
2-keto-D-gluconic acid	182	255	5.3	48
2-deoxy-D-glucose	181	227	4.8	47
D-glucuronic acid lactone	165	218	5.0	44
D-glucuronic acid (Na)	173	241	5.6	43
L-gulono-γ-lactone	152	195	5.0	39
L-sorbosone	178	160	4.7	34
D-glucono-δ-lactone	130	190	5.7	33
D-galacturonic acid	130	180	6.0	30

These cells converted L-galactose, L-galactono-γ-lactone and D-mannose to AA more rapidly than they did glucose, suggesting that mannose exerts its effect in the biosynthetic pathway "downstream" from glucose.

Example 5

Using the methods described above, a collection of mutants was assembled. The specific AA formation for representative mutants are shown in Table 5. The genealogy of these isolates is presented graphically in the "family tree" of Fig. 3.

These isolates were tested for their ability to convert compounds which could be converted to AA by strain UV77-247. Testing was done as in Example 4. Results are shown in Table 9.

TABLE 9

Conversion of Compounds to AA by Resting Cells of Mutant Strains of *Prototheca* of Varying Abilities to Synthesize AA

Strain	Absolute AA, mg/L					
	Buffer	Glucose	L-galactose	L-gal-γ-lact.	Mannose	Fructose
EMS13-4	53	97	191	173	139	ND
UV127-10	45	140	213	140	128	143
SP2-3	19	19	204	146	24	27
NA21-14	61	80	147	158	118	115
UV82-21	15	16	183	175	18	17
UV213-1	16	15	170	135	17	16
UV218-1	16	18	136	176	19	21
UV244-1	16	16	164	162	16	16
UV244-15	26	77	30	21	94	89
UV244-16	28	64	53	53	53	66

ND = Not Determined

These data suggest that the mutational blocks in those strains which convert fructose and mannose to AA poorly are before ("upstream" from) L-galactose and L-galactono-γ-lactone in the pathway.

Example 6

The following example shows that magnesium inhibits early steps in the production of AA.

To address the question of whether magnesium actually inhibits AA synthesis, strain NA45-3 (ATCC 209681) was grown in magnesium (Mg)-limited and Mg-sufficient medium. ATCC No. 209681, identified as *Prototheca moriformis* NA45-3 (Source:

repeated mutagenesis of ATCC No. 75669; Eucaryotic alga. Division Chlorophyta, Class Chlorophyceae, Order Chlorococcales), was deposited on March 13, 1998, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Cells from both cultures were harvested and resuspended in the cell-free supernate from the Mg-limited culture, and to half of each cell suspension additional magnesium was added in order to bring the level in the suspension to the Mg-sufficient level. The four conditions under which assays were run were as follows.

TABLE 10

Conditions Used to Test the Effect of Magnesium on AA Production

Condition	Magnesium concentration, g/L, during:	
	Growth	Assay
1Mg>1Mg	0.02	0.02
1Mg>10Mg	0.02	0.2
10Mg>1Mg	0.2	0.02
10Mg>10Mg	0.2	0.2

Substrates previously shown to lead to the formation of AA, namely D-glucose, D-glucosone, D-fructose. D-galactose, D-mannose, and L-galactono-γ-lactone, were added at 20 g/L to the four cell suspensions. Accumulation of AA after 24 hours was measured and compared to a control in which no substrate was added. The results of this study are shown graphically in Fig. 4.

When cells growing under magnesium-limited conditions were incubated with substrates in low-magnesium broth (1Mg>1Mg condition), all showed significant and similar accumulation of AA over the control condition. When the same cells were incubated in high magnesium broth (1Mg>10Mg condition), the accumulation of AA was reduced about 40% for all substrates except D-mannose and L-galactono-γ-lactone, suggesting that 1) the rate-limiting step in the conversion of D-glucose, D-glucosone, D-fructose, and D-galactose to AA is inhibited by magnesium or 2) magnesium stimulates an enzyme which results in the conversion of these compounds to some other compound(s), reducing the amount of substrate available for AA synthesis. On the other

hand, conversion of D-mannose and L-galactono-γ-lactone appeared to be unaffected by the presence of magnesium in the resuspension buffer, indicating that either 1) magnesium-inhibited enzymes are not involved in the conversion of these substrates to AA or 2) D-mannose and L-galactono-γ-lactone enter the pathway far enough downstream from the point where they can be siphoned off by side reactions involving Mg-requiring enzymes.

When cells were grown under magnesium-sufficient conditions, very little AA accumulation from any of the D-sugars was observed, regardless of the level of magnesium in the resuspension broth. Accumulation of AA from L-galactono-γ-lactone, however, was enhanced over that observed when cells are grown in Mg-limited conditions. This suggests that enzymes early in the pathway are repressed under Mg-sufficient conditions. Thus, the D-substrates all behaved similarly, with the exception of the apparent lack of magnesium inhibition of D-mannose conversion to AA. This would suggest that D-mannose enters the AA biosynthetic pathway at a point other than the other D-sugars.

Figs. 2A and 2B represent some of the fates of glucose in plants. The first enzymatic step in this scheme which commits carbon to glycolysis is the conversion of fructose-6-P to fructose-1,6-diP by phosphofructokinase (PFK). This reaction is essentially irreversible, and leads to the well known TCA cycle and oxidative phosphorylation, with concomitant ATP and NADH/NADPH generation. PFK has an absolute requirement for magnesium. If magnesium is limiting, this reaction could slow and eventually stop, blocking the flow of carbon through glycolysis and beyond, and would result in cessation of cell division even in the presence of excess glucose. One would expect fructose-6-P to accumulate under these conditions, fueling AA synthesis by the pathway shown in Figs. 1 and 2.

Example 7

The following example shows the correlation in *Prototheca* between AA production and the activity levels of the enzymes in the AA pathway.

Phosphomannose isomerase (PMI) Assay

PMI activity was first assayed (See Fig. 1). Ten strains representing a range of AA productivities were grown according to the standard protocol to measure AA-synthesizing ability. Cells were harvested 96 hours into magnesium-limited incubation, washed and resuspended in buffer containing 50 mM Tris/10 mM $MgCl_2$, pH 7.5. The suspended cells were broken in a French press, spun at 30,000 x g for 30 minutes, and desalted through Sephadex G-25 (Pharmacia PD-10 columns). Reactions were carried out in the reverse direction by adding various volumes of extracts to solutions of Tris/Mg buffer containing 0.15 U phosphoglucose isomerase (EC 5.3.1.9), 0.5 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and 1.0 mM NADP. Reactions were initiated by addition of 3 mM (final) mannose-6-phosphate. Final reaction volume was 1.0 mL. All components were dissolved in Tris/Mg buffer. Activities were taken as the change in A_{340}/min. From these activities was subtracted the activities measured in identical reaction mixtures lacking the M-6-P substrate. Specific activities were calculated by normalizing the activities for protein concentration in the reactions. Protein in the original extracts was determined by the method of Bradford, using a kit from Bio-Rad Laboratories (Hercules, CA). All enzymes and nucleotides were purchased from Sigma Chemical Co. (St. Louis, MO).

Phosphomannomutase (PMM) Assay

Phosphomannomutase was measured in a similar manner in the same strains, but these assay reaction mixtures also contained 0.25 mM glucose-1,6-diphosphate, 0.5 U commercially available PMI, and the reactions were started with the addition of 3.0 mM (final) mannose-1-phosphate rather than mannose-6-phosphate.

Phosphofructokinase (PFK) Assay

To shed light on the possibility that the enhancement of AA concentration in cultures which were limited for magnesium was due to a diversion of carbon from normal metabolism by a reduced activity of the first committed step in glycolysis (PFK) the strains were also assayed to confirm the presence of this enzyme activity. Cells were cultured, washed and broken as above. Extracts were centrifuged at 100,000 x g for 90 min before

desalting. Reactions were carried out in the forward direction by adding various volumes of extracts to solutions of Tris/Mg buffer containing 1.5 mM dithiothreitol, 0.86 U aldolase (EC 4.1.2.13), 1.4 U α-glycerophosphate dehydrogenase (EC 1.1.1.8), 14 U triosephosphate isomerase (EC 5.3.1.1), 0.11 mM NADH, and 1.0 mM ATP. Reactions were initiated by addition of 5 mM (final) fructose-6-phosphate. Final reaction volume was 1.0 mL. All components were dissolved in Tris/Mg buffer. Activities were taken as the change in A_{340}/min. From these activities were subtracted the activities measured in identical reaction mixtures lacking the F-6-P substrate. Specific activities were calculated by normalizing the activities for protein concentration in the reaction. Protein in the original extracts was determined as above.

GDP-D-mannose pyrophosphorylase (GMP) Assay

These same mutant strains were assayed for the next enzyme in the proposed pathway, GMP. Strains were grown both according to the standard Mg-limiting protocol (harvested 43-48 hours into magnesium-limited incubation) and in standard Mg-sufficient medium (harvesting all cells before glucose depletion). Washed cell pellets were resuspended in 50 mM phosphate buffer, pH 7.0, containing 20% (v/v) glycerol and 0.1 M sodium chloride (3 mL buffer/g wet cells), and broken in a French press. Crude extracts were spun at 15,000 x g for 15 minutes. Reactions were carried out in the forward direction by adding various volumes of extracts to solutions of 50 mM phosphate/4 mM $MgCl_2$ buffer, pH 7.0, containing 1 mM GTP. Reactions were initiated by addition of 1 mM (final) mannose-1-phosphate. Final reaction volume was 0.1 mL. Reaction mixtures were incubated at 30 C for 10 min, filtered through a 0.45 μm PVDF syringe filter, and analyzed for GDP-mannose by HPLC. A Supelcosil SAX1 column (4.6 x 250 mm) was used with a solvent gradient (1 mL/min) of: A - 6 mM potassium phosphate, pH 3.6; B - 500 mM potassium phosphate, pH 4.5. The gradient was: 0-3 min, 100% A; 3-10 min, 79% A; 10-15 min, 29% A. Column temperature was 30 C. Two assays that showed enzyme activity proportional to the amount of protein were averaged. Control no-substrate and no-extract reactions were also run. Specific activity was calculated by normalizing the activity for protein concentration in the reaction. Protein in the original extracts was determined as above.

GDP-D-mannose:GDP-L-galactose Epimerase Assay

Further tests measured the activities of the next enzyme in the proposed pathway, GDP-D-mannose:GDP-L-galactose epimerase. Strains were grown according to the standard protocol, harvested 43-48 hours into magnesium-limited incubation, washed, and resuspended in buffer containing 50 mM MOPS/5 mM EDTA, pH 7.2. Washed pellets were broken in a French press, and spun at 20,000 x g for 20 min. Protein determinations were made as above and a dilution series of each was made, ranging from 0.4 to 2.2 mg protein/mL. 50 µL aliquots of these dilutions were added to 10 µL aliquots of 6.3 mM GDP-D-mannose in which a portion of this substrate was universally labeled with ^{14}C in the mannose moiety. This substrate had an activity of 16 µCi/mL before dilution into the reaction mixture. Reactions were stopped after 10 min by transferring 20 µL of the mixture into microfuge tubes containing 20 µL of 250 mM trifluoroacetic acid (TFA) containing 1.0 g/L each D-mannose and L-galactose. These tubes were sealed and boiled for 10 min, cooled, spun for 60 sec in a Beckman Microfuge E, and 5 µL of each hydrolysate was spotted on 20 x 20 cm plastic-backed EM Science Silica gel 60 thin-layer chromatography plates (#5748/7), with 1 cm lanes created by scoring with a blunt stylus. After drying, plates were twice chromatographed for 2.5 hours in ethyl acetate:isopropanol:water, (65:22.3:12.7) (plates were dried between runs). Spots of free sugars were visualized by spraying dried plates with 0.5% p-anisaldehyde in a 62% ethanolic solution of 0.89 M sulfuric acid and 0.17 mM glacial acetic acid, and heating at 105 C for about 15 min. Spots of L-galactose and D-mannose were cut from the plates and counted in a scintillation counter (Beckman model 2800). For time-zero control counts, 16.7 µL of each extract dilution was added to 23.3 µL of the labeled substrate above, which had been diluted 1:7 with the TFA/mannose/galactose solution.

Table 11 summarizes the results of the five enzyme assays for the strains tested, along with their specific AA formations.

TABLE 11

Specific Enzyme Activities (mU)* of Selected Mutant *Prototheca* Strains

Strain	AA Specific Form, mg/g	PMI	PMM	PFK	GMP		Epimerase
					Mg-limited	Mg-sufficient	
UV164-6	78.4						0.79
EMS13-4	73.7	10.8	69.6	13.5	2.6	6.8	0.78
UV140-1	69.9						0.78
NA45-3	61.4						0.58
UV77-247	44.4						0.52
UV127-10	40.1	11.1	45.8	24.4	4.3	5.9	0.39
UV244-15	24.5	14.3	41.5		3.1	5.3	0.42
NA21-14	23.6	12.1	60.3	47.4	2.4	7.6	0.27
ATCC 75669	21.9						0.28
UV244-16	5.0	16.5	85.6		4.3	5.2	
SP2-3	2.0	17.7	47.0	64.5	2.0	7.5	0.03
UV218-1	0.4	15.9	72.1		2.7	7.0	0.83
UV213-1	0.1	19.7	47.7	32.6	3.2	6.7	0.60
UV82-21	0.0	14.6	70.6	30.4	4.1	7.5	0.15
UV244-1	0.0	18.2	51.1		5.5	12	0.15

Units: PMI and PMM, nmoles NADP reduced per min/mg protein; PFK, nmoles NADH oxidized per min/mg protein; GMP, nmoles GDP-D-mannose formed per min/mg protein; epimerase, nmoles GDP-L-galactose formed per min/mg protein.

The only enzyme which showed a strong correlation between activity and the ability to synthesize AA was the GDP-D-mannose:GDP-L-galactose epimerase. This correlation is depicted in Fig. 5. All of the strains which produced measurable amounts of AA had measurable amounts of epimerase activity. The converse was not true: four of the strains which synthesize little or no AA had significant epimerase activities. These strains are candidates for having mutations which affect enzymatic steps downstream from the epimerase. Since all of the strains tested can synthesize AA from L-galactose and L-galactono-γ-lactone (see Examples 4 and 5), the genetic lesion(s) in these four mutants must lie between GDP-L-galactose and free L-galactose.

Example 8

The next example shows the relationship between GDP-D-mannose:GDP-L-galactose epimerase activity and the degree of magnesium limitation in two strains, the original unmutagenized parent strain ATCC 75669, and one of the best AA producers, EMS13-4 (ATCC _____).

Four flasks of each strain were grown according to the standard protocol. One culture of each was harvested 24 hours into magnesium-limited incubation, and every 24 hours thereafter for a total of four days. One flask of each strain was also harvested 24 hours into magnesium sufficient incubation. All cultures had glucose remaining when harvested. Fig. 6 shows graphically the AA productivity and epimerase activity in EMS13-4 and ATCC 75669 as the cultures became Mg-limited. Epimerase activity in EMS13-4 was significantly greater than that in ATCC 75669 at all time points. There was also a concurrent rapid rise in both AA productivity and epimerase activity in EMS13-4 as the cultures became increasingly Mg-limited. While there was a moderate increase in AA productivity in ATCC 75669 as Mg became more limiting, there was no effect on epimerase activity.

Example 9

The following example shows the results of epimerase assays performed with extracts of two *E. coli* strains into which were cloned the *E. coli* gene for GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The *E. coli* K12 *wca* gene cluster is responsible for cholanic acid production; *wcaG* encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The *E. coli wcaG* sequence (nucleotides 4 through 966 of SEQ ID NO:3) was amplified by PCR from *E. coli* W3110 genomic DNA using primers WG EcoRI 5 (5' TAGAATTCAGTAAACAACGAGTTTTTATTGCTGG 3'; SEQ ID NO:12) and WG XhoI 3 (5' AACTCGAGTTACCCCCAAAGCGGTCTTGATTC 3'; SEQ ID NO:13). The 973-bp PCR product was ligated into the vector pPCR-Script SK(+) (Stratagene, LaJolla, CA). The 973-bp ExoRII/XhoI fragment was moved from this plasmid into the ExoRII/XhoI sites of pGEX-5X-1 (Amersham Pharmacia Biotech, Piscataway, NJ), creating plasmid pSW67-1. Plasmid pGEX-5X-1 is a GST gene fusion vector which adds a 26-kDa GST moiety onto the N-terminal end of the protein of interest. *E. coli* BL21(DE3) was transformed with pSW67-1 and pGEX-5X-1, resulting in strains BL21(DE3)/pSW67-1 and BL21(DE3)/pGEX-5X-1.

The *E. coli wcaG* sequence (nucleotides 1 through 966 of SEQ ID NO:3) was also amplified by PCR from *E. coli* W3110 genomic DNA using primers WG EcoRI 5-2 (5' CTGGAGTCGAATTCATGAGTAAACAACGAG 3'; SEQ ID NO:14) and WG PstI 3

(5' AACTGCAGTTACCCCCGAAAGCGGTCTTGATTC 3'; SEQ ID NO:15). The 976-bp PCR product was ligated into a pPCR-Script (Stratagene). The 976-bp ExoRII/PstI fragment was moved from this plasmid into the ExoRII/PstI sites of expression vector pKK223-3 (Amersham Pharmacia Biotech), creating plasmid pSW75-2. *E. coli* JM105 was transformed with pKK223-3 and pSW75-2, resulting in strains JM105/pKK223-3 and JM105/pSW75-2.

All six strains were grown in duplicate at 37°C with shaking in 2X YTA medium until an optical density of 0.8-1.0 at 600 nm was reached (about three hours). 2X YTA contains 16 g/L tryptone, 10 g/L yeast extract, 5 g/L sodium chloride and 100 mg/L ampicillin. One of each culture was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to 1 mM final concentration. All 12 cultures were incubated for an additional four hours, washed in 0.9% NaCl, and the cells were frozen at -80°C. Prior to pelleting the cells for preparation of extracts, a portion of each culture was used for a plasmid DNA miniprep to confirm the presence of the appropriate plasmids in these strains. A protein preparation of each culture was also run on SDS gels to confirm expression of a protein of the appropriate size where expected. Frozen pellets were thawed, resuspended in 2.5 mL MOPS/EDTA buffer, pH 7.2, broken in a French Press (10,000 psi), spun for 20 min at 20,000 x g, assayed for protein as above and diluted to 0.01, 0.1, 1.0 and 3 mg/mL protein.

Induction of the strain BL21(DE3)/pGEX-5X-1 resulted in high-level expression of a 26-kDa protein indicating the synthesis of the native GST protein. Induction of strain BL21(DE3)/pSW67-1 resulted in high-level expression of a 62-kDa protein, indicating the synthesis of the native GST protein (26K) fused to the *wcaG* gene product (36K). An aliquot of the fusion protein was treated with the protease Factor Xa (New England Biolabs, Beverly, MA), which cleaves near the GST/*wcaG* junction. Induction of the strain JM105/pSW75-2 resulted in high level expression of a 36-kDa protein, indicating the synthesis of the *wcaG* gene product. No such protein was detected in JM105/pKK223-3 (vector only).

Next, it was of interest to test extracts in the standard epimerase assay described in Example 7 to determine if any of the extracts containing the *wcaG* product could bring

about the conversion of GDP-D-mannose to GDP-L-galactose. The extracts to be assayed are:

BL21(DE3) Group

1. BL21(DE3) uninduced
2. BL21(DE3) induced with 1mM IPTG
3. BL21(DE3)/pGEX-5X-1 uninduced
4. BL21(DE3)/pGEX-5X-1 induced with 1mM IPTG
5. BL21(DE3)/pSW67-1 uninduced
6. BL21(DE3)/pSW67-1 induced with 1 mM IPTG; fusion protein intact
7. BL21(DE3)/pSW67-1 induced with 1 mM IPTG; GST moiety cleaved

JM105 Group

1. JM105 uninduced
2. JM105 induced with 1mM IPTG
3. JM105/pKK223-3 uninduced
4. JM105/pKK223-3 induced with 1 mM IPTG
5. JM105/pSW75-2 uninduced
6. JM105/pSW75-2 induced with 1 mM IPTG

Extracts 1 and 7 from the BL21(DE3) group and extracts 1 and 6 from the JM105 group were tested for GDP-D-mannose:GDP-L-galactose epimerase-like activity in a pilot experiment. In this initial experiment, no epimerase activity was detected in any of the extracts. At this time, such a result can be attributed to a number of possibilities. First, it is possible that the *wcaG* gene product is incapable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose, although this conclusion can not be reached until several other parameters are tested. Second, it is possible that under the assay conditions which are satisfactory to measure activity for the endogenous GDP-D-mannose:GDP-L-galactose epimerase, the *wcaG* gene product does not have GDP-D-mannose:GDP-L-galactose epimerase-like activity. Therefore, alternate conditions should be tested. Additionally, confirmation experiments should be performed to confirm the accuracy of the pilot conditions. Third, although the BL21(DE3) and the JM105 clones produce proteins of the expected size, the constructs have not been sequenced to confirm the proper coding sequence for the *wcaG* gene product and thereby rule out PCR or cloning errors which may render the *wcaG* gene product inactive. Fourth, the protein formed from the cloned sequence is full-length, but inactive, for example, due to incorrect tertiary structure (folding). Fifth, the gene is overexpressed, resulting in accumulation of insoluble and inactive protein products (inclusion bodies). Future experiments will attempt to

determine whether the constructs have or can be induced to have the ability to catalyze the conversion of GDP-D-mannose to GDP-L-galactose, and to use the sequences to isolate the endogenous GDP-D-mannose:GDP-L-galactose epimerase.

Table 12 provides the atomic coordinates for Brookhaven Protein Data Bank Accession Code 1bws:

TABLE 12

```
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TITLE     CRYSTAL STRUCTURE OF GDP-4-KETO-6-DEOXY-D-MANNOSE
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TITLE    3 THE BIOSYNTHESIS OF GDP-L-FUCOSE
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COMPND    2 MOLECULE: GDP-4-KETO-6-DEOXY-D-MANNOSE EPIMERASE/REDUCTASE;
COMPND    3 CHAIN: A;
COMPND    4 ENGINEERED: YES;
COMPND    5 BIOLOGICAL_UNIT: HOMODIMER
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SOURCE    3 EXPRESSION_SYSTEM: ESCHERICHIA COLI
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EXPDTA    X-RAY DIFFRACTION
AUTHOR    DE M.RIZZITONETTIFLORA
REVDAT    1   13-JAN-99 1BWS     0
JRNL        AUTH   DE D.RIZZITONETTIVIGEVANISTURLABISSOFLORA
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JRNL        TITL 2 FROM ESCHERICHIA COLI, A KEY ENZYME IN THE
JRNL        TITL 3 BIOSYNTHESIS OF GDP-L-FUCOSE, DISPLAYS THE
JRNL        TITL 4 STRUCTURAL CHARACTERISTICS OF THE RED PROTEIN
JRNL        TITL 5 HOMOLOGY SUPERFAMILY
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JRNL        REFN                                        9999
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REMARK 290            MMM -> TRANSLATION VECTOR
REMARK 290
REMARK 290 CRYSTALLOGRAPHIC SYMMETRY TRANSFORMATIONS
REMARK 290 THE FOLLOWING TRANSFORMATIONS OPERATE ON THE ATOM/HETATM
REMARK 290 RECORDS IN THIS ENTRY TO PRODUCE CRYSTALLOGRAPHICALLY
REMARK 290 RELATED MOLECULES.
REMARK 290   SMTRY1   1  1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2   1  0.000000  1.000000  0.000000        0.00000
REMARK 290   SMTRY3   1  0.000000  0.000000  1.000000        0.00000
REMARK 290   SMTRY1   2 -0.500045 -0.865974  0.000000        0.00000
REMARK 290   SMTRY2   2  0.866077 -0.499955  0.000000        0.00000
REMARK 290   SMTRY3   2  0.000000  0.000000  1.000000       50.58553
REMARK 290   SMTRY1   3 -0.499955  0.865974  0.000000        0.00000
REMARK 290   SMTRY2   3 -0.866077 -0.500045  0.000000        0.00000
REMARK 290   SMTRY3   3  0.000000  0.000000  1.000000       25.29276
REMARK 290   SMTRY1   4 -0.500045  0.865922  0.000000        0.00000
REMARK 290   SMTRY2   4  0.866077  0.500045  0.000000        0.00000
REMARK 290   SMTRY3   4  0.000000  0.000000 -1.000000        0.00000
REMARK 290   SMTRY1   5  1.000000  0.000104  0.000000        0.00000
REMARK 290   SMTRY2   5  0.000000 -1.000000  0.000000        0.00000
REMARK 290   SMTRY3   5  0.000000  0.000000 -1.000000       25.29276
REMARK 290   SMTRY1   6 -0.499955 -0.866026  0.000000        0.00000
REMARK 290   SMTRY2   6 -0.866077  0.499955  0.000000        0.00000
REMARK 290   SMTRY3   6  0.000000  0.000000 -1.000000       50.58553
REMARK 290
REMARK 290 REMARK: NULL
REMARK 465
REMARK 465 MISSING RESIDUES
REMARK 465 THE FOLLOWING RESIDUES WERE NOT LOCATED IN THE
REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE):
REMARK 465
REMARK 465   M RES C SSSEQI
REMARK 465     MET A     1
REMARK 465     SER A     2
REMARK 465     ASP A   317
REMARK 465     ARG A   318
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REMARK 465     PHE A   319
REMARK 465     ARG A   320
REMARK 465     GLY A   321
REMARK 800
REMARK 800 SITE
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
DBREF  1BWS A    3   316  SWS    P32055   FCL_ECOLI
SEQRES   1 A  321  MET SER LYS GLN ARG VAL PHE ILE ALA GLY HIS ARG GLY
SEQRES   2 A  321  MET VAL GLY SER ALA ILE ARG ARG GLN LEU GLU GLN ARG
SEQRES   3 A  321  GLY ASP VAL GLU LEU VAL LEU ARG THR ARG ASP GLU LEU
SEQRES   4 A  321  ASN LEU LEU ASP SER ARG ALA VAL HIS ASP PHE PHE ALA
SEQRES   5 A  321  SER GLU ARG ILE ASP GLN VAL TYR LEU ALA ALA ALA LYS
SEQRES   6 A  321  VAL GLY GLY ILE VAL ALA ASN ASN THR TYR PRO ALA ASP
SEQRES   7 A  321  PHE ILE TYR GLN ASN MET MET ILE GLU SER ASN ILE ILE
SEQRES   8 A  321  HIS ALA ALA HIS GLN ASN ASP VAL ASN LYS LEU LEU PHE
SEQRES   9 A  321  LEU GLY SER SER CYS ILE TYR PRO LYS LEU ALA LYS GLN
SEQRES  10 A  321  PRO MET ALA GLU SER GLU LEU LEU GLN GLY THR LEU GLU
SEQRES  11 A  321  PRO THR ASN GLU PRO TYR ALA ILE ALA LYS ILE ALA GLY
SEQRES  12 A  321  ILE LYS LEU CYS GLU SER TYR ASN ARG GLN TYR GLY ARG
SEQRES  13 A  321  ASP TYR ARG SER VAL MET PRO THR ASN LEU TYR GLY PRO
SEQRES  14 A  321  HIS ASP ASN PHE HIS PRO SER ASN SER HIS VAL ILE PRO
SEQRES  15 A  321  ALA LEU LEU ARG ARG PHE HIS GLU ALA THR ALA GLN ASN
SEQRES  16 A  321  ALA PRO ASP VAL VAL VAL TRP GLY SER GLY THR PRO MET
SEQRES  17 A  321  ARG GLU PHE LEU HIS VAL ASP ASP MET ALA ALA ALA SER
SEQRES  18 A  321  ILE HIS VAL MET GLU LEU ALA HIS GLU VAL TRP LEU GLU
SEQRES  19 A  321  ASN THR GLN PRO MET LEU SER HIS ILE ASN VAL GLY THR
SEQRES  20 A  321  GLY VAL ASP CYS THR ILE ARG ASP VAL ALA GLN THR ILE
SEQRES  21 A  321  ALA LYS VAL VAL GLY TYR LYS GLY ARG VAL VAL PHE ASP
SEQRES  22 A  321  ALA SER LYS PRO ASP GLY THR PRO ARG LYS LEU LEU ASP
SEQRES  23 A  321  VAL THR ARG LEU HIS GLN LEU GLY TRP TYR HIS GLU ILE
SEQRES  24 A  321  SER LEU GLU ALA GLY LEU ALA SER THR TYR GLN TRP PHE
SEQRES  25 A  321  LEU GLU ASN GLN ASP ARG PHE ARG GLY
HET    NDP      1       0
HETNAM     NDP NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE
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HETSYN     NDP NADP
FORMUL   2  NDP    C21 H23 N7 O17 P3 3-
FORMUL   3  HOH   *109(H2 O1)
HELIX    1   1 MET A   14  GLN A   25  1                                  12
HELIX    2   2 SER A   44  GLU A   54  1                                  11
HELIX    3   3 ILE A   69  THR A   74  1                                   6
HELIX    4   4 PRO A   76  ASN A   97  1                                  22
HELIX    5   5 SER A  108  ILE A  110  5                                   3
HELIX    6   6 GLU A  121  GLU A  123  5                                   3
HELIX    7   7 GLU A  134  TYR A  154  1                                  21
HELIX    8   8 VAL A  180  ALA A  193  1                                  14
HELIX    9   9 VAL A  214  GLU A  226  1                                  13
HELIX   10  10 HIS A  229  GLU A  234  1                                   6
HELIX   11  11 ILE A  253  VAL A  264  1                                  12
HELIX   12  12 THR A  288  GLN A  292  1                                   5
HELIX   13  13 LEU A  301  GLU A  314  1                                  14
SHEET    1   A 6 VAL A  29  VAL A  32  0
SHEET    2   A 6 GLN A   4  ALA A   9  1  N  GLN A   4   O  GLU A  30
SHEET    3   A 6 GLN A  58  LEU A  61  1  N  GLN A  58   O  PHE A   7
SHEET    4   A 6 LYS A 101  LEU A 105  1  N  LYS A 101   O  VAL A  59
SHEET    5   A 6 ASP A 157  PRO A 163  1  N  ASP A 157   O  LEU A 102
SHEET    6   A 6 ILE A 243  VAL A 245  1  N  ILE A 243   O  MET A 162
SHEET    1   B 2 ASN A 165  TYR A 167  0
SHEET    2   B 2 PHE A 211  HIS A 213  1  N  LEU A 212   O  ASN A 165
SHEET    1   C 2 ASP A 198  TRP A 202  0
SHEET    2   C 2 ARG A 269  ASP A 273  1  N  ARG A 269   O  VAL A 199
SITE     1 CAT  1 TYR  136
SITE     2 CAT  1 LYS  140
SITE     3 CAT  1 SER  107
CRYST1  104.200  104.200   75.880  90.00  90.00 120.00 P 32 2 1      6
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.009597  0.005541  0.000000        0.00000
SCALE2      0.000000  0.011081  0.000000        0.00000
SCALE3      0.000000  0.000000  0.013179        0.00000
HETATM   1  O   HOH     1      55.652 -16.806  22.535  1.00  8.73           O
HETATM   2  O   HOH     3      58.494 -10.639  18.740  1.00 13.17           O
HETATM   3  O   HOH     4      58.230 -11.715  27.770  1.00 19.07           O
HETATM   4  O   HOH     5      57.252  -3.759  30.107  1.00 11.21           O
HETATM   5  O   HOH     6      58.298 -10.011  25.527  1.00 15.74           O
HETATM   6  O   HOH     7      49.321   6.583  38.815  1.00 19.33           O
HETATM   7  O   HOH     8      53.785  -4.262  22.464  1.00 10.94           O
HETATM   8  O   HOH    10      74.652   2.888   9.141  1.00 17.80           O
HETATM   9  O   HOH    11      49.761   0.826  32.896  1.00 22.02           O
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HETATM	10	O	HOH	12	55.530	-11.162	28.526	1.00	11.39	O
HETATM	11	O	HOH	13	75.027	7.034	27.353	1.00	16.30	O
HETATM	12	O	HOH	14	49.994	-2.314	11.032	1.00	21.33	O
HETATM	13	O	HOH	15	61.323	-8.959	29.657	1.00	22.84	O
HETATM	14	O	HOH	16	61.029	-11.560	29.131	1.00	21.24	O
HETATM	15	O	HOH	17	50.684	5.881	10.130	1.00	15.88	O
HETATM	16	O	HOH	18	64.506	-6.302	32.989	1.00	21.05	O
HETATM	17	O	HOH	19	57.856	-16.398	25.085	1.00	22.86	O
HETATM	18	O	HOH	20	38.979	26.536	19.070	1.00	21.08	O
HETATM	19	O	HOH	21	38.042	33.487	21.909	1.00	19.01	O
HETATM	20	O	HOH	24	38.172	35.775	20.827	1.00	33.46	O
HETATM	21	O	HOH	25	70.916	-11.128	15.244	1.00	31.37	O
HETATM	22	O	HOH	26	54.205	19.360	28.396	1.00	35.76	O
HETATM	23	O	HOH	27	50.436	2.654	16.783	1.00	12.25	O
HETATM	24	O	HOH	28	69.692	19.108	38.979	1.00	49.77	O
HETATM	25	O	HOH	29	56.432	-8.877	19.303	1.00	22.52	O
HETATM	26	O	HOH	30	60.832	3.415	42.349	1.00	17.39	O
HETATM	27	O	HOH	31	53.889	-12.706	29.764	1.00	22.40	O
HETATM	28	O	HOH	32	37.887	26.373	28.058	1.00	18.09	O
HETATM	29	O	HOH	33	49.201	11.173	26.867	1.00	33.95	O
HETATM	30	O	HOH	34	46.762	-0.278	31.394	1.00	20.63	O
HETATM	31	O	HOH	35	41.731	27.568	43.302	1.00	27.39	O
HETATM	32	O	HOH	36	66.827	11.202	28.929	1.00	13.23	O
HETATM	33	O	HOH	37	46.834	14.396	40.819	1.00	46.02	O
HETATM	34	O	HOH	38	61.342	1.064	43.868	1.00	26.68	O
HETATM	35	O	HOH	42	70.597	16.422	37.837	1.00	19.26	O
HETATM	36	O	HOH	44	72.275	-9.089	33.407	1.00	22.11	O
HETATM	37	O	HOH	45	42.685	34.461	33.955	1.00	17.32	O
HETATM	38	O	HOH	46	53.480	13.394	38.364	1.00	20.19	O
HETATM	39	O	HOH	47	56.085	21.757	44.744	1.00	33.50	O
HETATM	40	O	HOH	48	35.741	32.691	23.517	1.00	19.49	O
HETATM	41	O	HOH	49	40.458	36.700	34.312	1.00	34.53	O
HETATM	42	O	HOH	50	75.440	7.267	29.948	1.00	18.07	O
HETATM	43	O	HOH	51	47.476	18.347	20.851	1.00	34.16	O
HETATM	44	O	HOH	53	52.837	-16.344	19.587	1.00	25.92	O
HETATM	45	O	HOH	55	46.415	9.073	20.108	1.00	31.91	O
HETATM	46	O	HOH	57	45.912	35.170	36.133	1.00	35.55	O
HETATM	47	O	HOH	58	60.247	-2.880	41.919	1.00	16.85	O
HETATM	48	O	HOH	60	64.974	6.086	24.501	1.00	32.16	O
HETATM	49	O	HOH	61	52.103	4.683	4.978	1.00	35.72	O
HETATM	50	O	HOH	62	50.888	40.154	36.463	1.00	38.35	O
HETATM	51	O	HOH	63	44.373	31.233	37.336	1.00	20.07	O
HETATM	52	O	HOH	64	57.280	27.757	42.451	1.00	21.74	O
HETATM	53	O	HOH	65	58.409	23.769	45.517	1.00	58.42	O
HETATM	54	O	HOH	66	68.690	-11.764	35.335	1.00	57.07	O

HETATM	55	O	HOH	67	42.746	25.153	23.465	1.00	27.05	O
HETATM	56	O	HOH	68	53.638	-16.457	32.292	1.00	31.71	O
HETATM	57	O	HOH	69	33.390	41.716	31.408	1.00	29.92	O
HETATM	58	O	HOH	70	57.768	17.897	42.434	1.00	25.75	O
HETATM	59	O	HOH	71	75.647	9.164	11.766	1.00	35.13	O
HETATM	60	O	HOH	72	62.032	33.292	44.749	1.00	46.18	O
HETATM	61	O	HOH	73	47.310	14.312	34.285	1.00	31.18	O
HETATM	62	O	HOH	74	79.660	-3.947	15.913	1.00	34.63	O
HETATM	63	O	HOH	75	46.929	5.343	4.550	1.00	23.14	O
HETATM	64	O	HOH	76	73.475	12.039	28.412	1.00	27.26	O
HETATM	65	O	HOH	77	46.297	-6.982	30.032	1.00	43.41	O
HETATM	66	O	HOH	78	68.528	-3.422	40.869	1.00	38.47	O
HETATM	67	O	HOH	79	62.080	-1.448	42.803	1.00	24.60	O
HETATM	68	O	HOH	80	65.330	18.150	40.726	1.00	41.00	O
HETATM	69	O	HOH	81	51.775	16.128	37.607	1.00	25.11	O
HETATM	70	O	HOH	83	54.266	28.682	43.313	1.00	27.61	O
HETATM	71	O	HOH	85	73.291	-15.479	20.603	1.00	37.54	O
HETATM	72	O	HOH	86	34.760	21.479	28.544	1.00	43.87	O
HETATM	73	O	HOH	87	37.326	24.131	29.677	1.00	24.47	O
HETATM	74	O	HOH	88	65.168	20.148	6.735	1.00	26.10	O
HETATM	75	O	HOH	89	59.196	12.089	13.630	1.00	25.24	O
HETATM	76	O	HOH	91	66.576	-6.235	40.279	1.00	43.11	O
HETATM	77	O	HOH	93	37.339	29.394	25.515	1.00	27.56	O
HETATM	78	O	HOH	94	52.339	-17.014	42.271	1.00	48.96	O
HETATM	79	O	HOH	95	40.511	32.927	31.717	1.00	22.46	O
HETATM	80	O	HOH	96	78.580	13.121	34.138	1.00	27.98	O
HETATM	81	O	HOH	97	65.090	15.704	34.876	1.00	18.96	O
HETATM	82	O	HOH	99	84.562	2.951	27.181	1.00	35.92	O
HETATM	83	O	HOH	100	50.386	9.761	9.646	1.00	23.18	O
HETATM	84	O	HOH	101	67.649	-0.851	38.764	1.00	24.99	O
HETATM	85	O	HOH	102	44.001	4.293	34.315	1.00	31.13	O
HETATM	86	O	HOH	103	59.386	-5.071	26.211	1.00	29.10	O
HETATM	87	O	HOH	104	77.364	4.745	41.506	1.00	35.32	O
HETATM	88	O	HOH	105	59.034	21.201	32.414	1.00	23.43	O
HETATM	89	O	HOH	106	42.463	34.698	14.327	1.00	38.86	O
HETATM	90	O	HOH	107	70.217	14.292	20.864	1.00	42.39	O
HETATM	91	O	HOH	108	76.999	8.130	25.862	1.00	32.91	O
HETATM	92	O	HOH	109	49.766	29.937	22.173	1.00	42.52	O
HETATM	93	O	HOH	110	72.473	13.536	38.823	1.00	33.32	O
HETATM	94	O	HOH	111	64.328	-12.084	38.608	1.00	37.99	O
HETATM	95	O	HOH	112	60.161	16.382	42.682	1.00	35.68	O
HETATM	96	O	HOH	113	47.602	13.639	27.016	1.00	26.01	O
HETATM	97	O	HOH	115	64.606	11.644	40.107	1.00	30.33	O
HETATM	98	O	HOH	116	61.231	-15.137	27.255	1.00	38.76	O
HETATM	99	O	HOH	117	65.324	-11.223	35.098	1.00	30.45	O

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HETATM  100  O   HOH   119      56.602  17.219  44.932  1.00 36.53           O
HETATM  101  O   HOH   120      37.564  19.860  23.135  1.00 31.27           O
HETATM  102  O   HOH   121      64.845   5.057  21.132  1.00 45.57           O
HETATM  103  O   HOH   123      63.391  16.801  26.898  1.00 38.46           O
HETATM  104  O   HOH   124      42.567   6.134  32.635  1.00 31.56           O
HETATM  105  O   HOH   125      72.485  13.236  35.059  1.00 29.61           O
HETATM  106  O   HOH   126      65.229   3.650  44.032  1.00 36.86           O
HETATM  107  O   HOH   127      37.089   7.148  31.083  1.00 39.58           O
HETATM  108  O   HOH   128      73.327  10.546  12.123  1.00 34.97           O
HETATM  109  O   HOH   129      74.450  10.299  26.598  1.00 30.80           O
HETATM  110 AO5* NDP A   1      67.524  13.055  26.692  1.00 36.42           O
HETATM  111 AC5* NDP A   1      68.089  12.297  25.614  1.00  9.30           C
HETATM  112 AC4* NDP A   1      69.601  12.124  25.858  1.00 27.73           C
HETATM  113 AO4* NDP A   1      70.193  11.258  24.848  1.00 22.87           O
HETATM  114 AC3* NDP A   1      70.484  13.390  25.873  1.00 17.83           C
HETATM  115 AO3* NDP A   1      71.192  13.436  27.066  1.00 16.11           O
HETATM  116 AC2* NDP A   1      71.373  13.220  24.626  1.00 11.46           C
HETATM  117 AO2* NDP A   1      72.623  13.886  24.655  1.00 31.96           O
HETATM  118 AC1* NDP A   1      71.510  11.702  24.656  1.00 19.02           C
HETATM  119  O3   NDP A   1      65.336  13.590  26.129  1.00 20.59           O
HETATM  120 NO5* NDP A   1      63.536  11.943  26.448  1.00 28.99           O
HETATM  121 NC5* NDP A   1      64.328  10.843  25.957  1.00 24.89           C
HETATM  122 NC4* NDP A   1      63.467   9.646  25.686  1.00 31.79           C
HETATM  123 NO4* NDP A   1      62.837   9.337  26.908  1.00 28.82           O
HETATM  124 NC3* NDP A   1      62.340   9.837  24.665  1.00 11.50           C
HETATM  125 NO3* NDP A   1      62.891   9.402  23.461  1.00 28.60           O
HETATM  126 NC2* NDP A   1      61.152   8.996  25.138  1.00 28.11           C
HETATM  127 NO2* NDP A   1      60.881   7.662  24.715  1.00 24.30           O
HETATM  128 NC1* NDP A   1      61.947   8.875  26.580  1.00 35.35           C
HETATM  129 AP2* NDP A   1      73.104  15.069  23.823  1.00 32.96           P
HETATM  130 AOP1 NDP A   1      74.500  15.308  24.308  1.00 37.84           O
HETATM  131 AOP2 NDP A   1      72.797  14.925  22.348  1.00 36.66           O
HETATM  132 AOP3 NDP A   1      72.163  16.217  23.958  1.00 31.97           O
HETATM  133  AP  NDP A   1      66.660  14.257  26.393  1.00 26.17          XX
HETATM  134  AO1 NDP A   1      66.886  14.795  25.047  1.00 15.31          XX
HETATM  135  AO2 NDP A   1      66.439  15.207  27.521  1.00 34.39          XX
HETATM  136  AN9 NDP A   1      71.820  11.224  23.353  1.00 13.63          XX
HETATM  137  AC8 NDP A   1      71.104  11.316  22.200  1.00 12.41          XX
HETATM  138  AN7 NDP A   1      71.758  10.835  21.161  1.00 15.71          XX
HETATM  139  AC5 NDP A   1      72.933  10.313  21.710  1.00 16.17          XX
HETATM  140  AC6 NDP A   1      74.053   9.657  21.140  1.00 31.35          XX
HETATM  141  AN6 NDP A   1      74.165   9.464  19.819  1.00 12.59          XX
HETATM  142  AN1 NDP A   1      75.078   9.280  21.942  1.00 17.56          XX
HETATM  143  AC2 NDP A   1      74.971   9.578  23.251  1.00 15.44          XX
HETATM  144  AN3 NDP A   1      74.027  10.302  23.889  1.00 24.82          XX
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HETATM  145  AC4 NDP A   1      73.036  10.653  23.047  1.00 17.48           XX
HETATM  146  NP  NDP A   1      64.183  13.106  27.191  1.00 25.47            N
HETATM  147  NO1 NDP A   1      63.142  14.169  27.253  1.00 28.69            N
HETATM  148  NO2 NDP A   1      64.837  12.643  28.492  1.00 24.32            N
HETATM  149  NN1 NDP A   1      60.598   9.775  27.109  1.00 23.63            N
HETATM  150  NC2 NDP A   1      60.143  10.905  26.442-99.00 78.36            N
HETATM  151  NC3 NDP A   1      59.070  11.648  27.007-99.00100.00            N
HETATM  152  NC7 NDP A   1      58.497  13.017  26.528-99.00100.00            N
HETATM  153  NO7 NDP A   1      59.358  13.703  25.972-99.00100.00            N
HETATM  154  NN7 NDP A   1      57.207  13.400  26.912-99.00 84.38            N
HETATM  155  NC4 NDP A   1      58.442  11.146  28.137-99.00100.00            N
HETATM  156  NC5 NDP A   1      58.912   9.963  28.754-99.00100.00            N
HETATM  157  NC6 NDP A   1      59.951   9.266  28.147-99.00100.00            N
ATOM    158  N   LYS A   3      76.227  -5.632  44.315  1.00 61.49            N
ATOM    159  CA  LYS A   3      76.152  -4.302  43.684  1.00 58.00            C
ATOM    160  C   LYS A   3      75.985  -4.421  42.171  1.00 52.79            C
ATOM    161  O   LYS A   3      76.921  -4.737  41.419  1.00 44.76            O
ATOM    162  CB  LYS A   3      77.359  -3.417  44.030  1.00 59.74            C
ATOM    163  CG  LYS A   3      77.011  -1.944  44.314  1.00 50.87            C
ATOM    164  CD  LYS A   3      78.208  -1.161  44.894  1.00 61.21            C
ATOM    165  CE  LYS A   3      77.855  -0.377  46.186  1.00100.00            C
ATOM    166  NZ  LYS A   3      78.857  -0.401  47.343  1.00 70.61            N
ATOM    167  N   GLN A   4      74.746  -4.242  41.747  1.00 45.15            N
ATOM    168  CA  GLN A   4      74.408  -4.326  40.347  1.00 37.18            C
ATOM    169  C   GLN A   4      74.983  -3.166  39.561  1.00 34.93            C
ATOM    170  O   GLN A   4      75.127  -2.050  40.087  1.00 28.48            O
ATOM    171  CB  GLN A   4      72.915  -4.445  40.221  1.00 34.65            C
ATOM    172  CG  GLN A   4      72.456  -5.854  40.584  1.00 31.82            C
ATOM    173  CD  GLN A   4      72.570  -6.788  39.405  1.00 79.25            C
ATOM    174  OE1 GLN A   4      72.165  -6.452  38.286  1.00100.00            O
ATOM    175  NE2 GLN A   4      73.206  -7.925  39.623  1.00 80.24            N
ATOM    176  N   ARG A   5      75.475  -3.495  38.375  1.00 27.16            N
ATOM    177  CA  ARG A   5      76.146  -2.546  37.483  1.00 39.16            C
ATOM    178  C   ARG A   5      75.191  -2.018  36.433  1.00 38.22            C
ATOM    179  O   ARG A   5      74.938  -2.698  35.438  1.00 32.44            O
ATOM    180  CB  ARG A   5      77.398  -3.163  36.826  1.00 41.76            C
ATOM    181  CG  ARG A   5      78.692  -2.954  37.663  1.00 37.34            C
ATOM    182  CD  ARG A   5      80.015  -3.236  36.876  1.00 32.99            C
ATOM    183  NE  ARG A   5      81.036  -2.203  37.125  1.00 25.71            N
ATOM    184  CZ  ARG A   5      81.617  -1.488  36.169  1.00 32.53            C
ATOM    185  NH1 ARG A   5      81.293  -1.704  34.904  1.00 40.07            N
ATOM    186  NH2 ARG A   5      82.516  -0.551  36.474  1.00100.00            N
ATOM    187  N   VAL A   6      74.743  -0.773  36.659  1.00 32.08            N
ATOM    188  CA  VAL A   6      73.715  -0.082  35.881  1.00 28.89            C
ATOM    189  C   VAL A   6      74.161   1.021  34.897  1.00 29.37            C
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ATOM	190	O	VAL	A	6	74.745	2.041	35.274	1.00	22.50	O
ATOM	191	CB	VAL	A	6	72.577	0.378	36.813	1.00	23.52	C
ATOM	192	CG1	VAL	A	6	71.366	0.960	36.006	1.00	20.29	C
ATOM	193	CG2	VAL	A	6	72.108	-0.852	37.644	1.00	18.45	C
ATOM	194	N	PHE	A	7	73.948	0.749	33.615	1.00	22.92	N
ATOM	195	CA	PHE	A	7	74.267	1.710	32.573	1.00	27.15	C
ATOM	196	C	PHE	A	7	72.975	2.423	32.192	1.00	20.24	C
ATOM	197	O	PHE	A	7	71.994	1.788	31.815	1.00	20.71	O
ATOM	198	CB	PHE	A	7	74.864	1.004	31.374	1.00	18.98	C
ATOM	199	CG	PHE	A	7	74.916	1.836	30.115	1.00	21.83	C
ATOM	200	CD1	PHE	A	7	75.521	3.087	30.108	1.00	19.36	C
ATOM	201	CD2	PHE	A	7	74.483	1.284	28.886	1.00	23.50	C
ATOM	202	CE1	PHE	A	7	75.614	3.828	28.902	1.00	27.52	C
ATOM	203	CE2	PHE	A	7	74.548	1.996	27.685	1.00	19.33	C
ATOM	204	CZ	PHE	A	7	75.128	3.255	27.673	1.00	18.59	C
ATOM	205	N	ILE	A	8	72.959	3.727	32.454	1.00	18.75	N
ATOM	206	CA	ILE	A	8	71.844	4.588	32.112	1.00	14.25	C
ATOM	207	C	ILE	A	8	72.337	5.351	30.909	1.00	11.22	C
ATOM	208	O	ILE	A	8	73.259	6.165	30.998	1.00	17.76	O
ATOM	209	CB	ILE	A	8	71.507	5.605	33.212	1.00	14.15	C
ATOM	210	CG1	ILE	A	8	71.356	4.949	34.582	1.00	8.24	C
ATOM	211	CG2	ILE	A	8	70.183	6.342	32.874	1.00	16.85	C
ATOM	212	CD1	ILE	A	8	71.091	5.961	35.707	1.00	10.32	C
ATOM	213	N	ALA	A	9	71.896	4.906	29.752	1.00	16.42	N
ATOM	214	CA	ALA	A	9	72.256	5.559	28.513	1.00	18.74	C
ATOM	215	C	ALA	A	9	71.530	6.913	28.511	1.00	28.45	C
ATOM	216	O	ALA	A	9	70.411	7.032	29.045	1.00	22.39	O
ATOM	217	CB	ALA	A	9	71.808	4.731	27.311	1.00	14.43	C
ATOM	218	N	GLY	A	10	72.199	7.922	27.940	1.00	20.06	N
ATOM	219	CA	GLY	A	10	71.706	9.284	27.911	1.00	18.62	C
ATOM	220	C	GLY	A	10	71.407	9.819	29.305	1.00	16.40	C
ATOM	221	O	GLY	A	10	70.379	10.448	29.481	1.00	17.36	O
ATOM	222	N	HIS	A	11	72.295	9.581	30.272	1.00	10.32	N
ATOM	223	CA	HIS	A	11	72.068	9.966	31.688	1.00	13.90	C
ATOM	224	C	HIS	A	11	72.008	11.504	31.916	1.00	21.52	C
ATOM	225	O	HIS	A	11	71.700	11.994	32.983	1.00	13.22	O
ATOM	226	CB	HIS	A	11	73.153	9.350	32.581	1.00	14.88	C
ATOM	227	CG	HIS	A	11	74.502	9.948	32.326	1.00	23.73	C
ATOM	228	ND1	HIS	A	11	75.239	9.648	31.197	1.00	24.90	N
ATOM	229	CD2	HIS	A	11	75.167	10.952	32.956	1.00	16.35	C
ATOM	230	CE1	HIS	A	11	76.317	10.407	31.170	1.00	22.54	C
ATOM	231	NE2	HIS	A	11	76.271	11.240	32.197	1.00	17.56	N
ATOM	232	N	ARG	A	12	72.310	12.288	30.908	1.00	22.31	N
ATOM	233	CA	ARG	A	12	72.147	13.693	31.122	1.00	18.90	C
ATOM	234	C	ARG	A	12	70.851	14.244	30.495	1.00	26.34	C

ATOM	235	O	ARG	A	12	70.572	15.426	30.604	1.00 25.37	O
ATOM	236	CB	ARG	A	12	73.352	14.418	30.587	1.00 25.93	C
ATOM	237	CG	ARG	A	12	74.582	13.943	31.279	1.00 53.87	C
ATOM	238	CD	ARG	A	12	75.757	14.619	30.699	1.00 32.53	C
ATOM	239	NE	ARG	A	12	76.359	15.576	31.605	1.00 69.90	N
ATOM	240	CZ	ARG	A	12	76.971	16.675	31.178	1.00100.00	C
ATOM	241	NH1	ARG	A	12	77.001	16.948	29.867	1.00100.00	N
ATOM	242	NH2	ARG	A	12	77.526	17.508	32.056	1.00100.00	N
ATOM	243	N	GLY	A	13	70.078	13.420	29.800	1.00 18.25	N
ATOM	244	CA	GLY	A	13	68.802	13.904	29.258	1.00 16.50	C
ATOM	245	C	GLY	A	13	67.849	14.144	30.428	1.00 18.88	C
ATOM	246	O	GLY	A	13	68.202	13.902	31.624	1.00 14.04	O
ATOM	247	N	MET	A	14	66.653	14.632	30.103	1.00 16.00	N
ATOM	248	CA	MET	A	14	65.688	14.981	31.128	1.00 13.49	C
ATOM	249	C	MET	A	14	65.293	13.760	31.901	1.00 14.02	C
ATOM	250	O	MET	A	14	65.408	13.713	33.145	1.00 17.06	O
ATOM	251	CB	MET	A	14	64.442	15.605	30.524	1.00 11.57	C
ATOM	252	CG	MET	A	14	63.320	15.628	31.559	1.00 20.77	C
ATOM	253	SD	MET	A	14	61.926	16.766	31.110	1.00 29.16	S
ATOM	254	CE	MET	A	14	62.527	17.108	29.574	1.00 30.68	C
ATOM	255	N	VAL	A	15	64.798	12.769	31.158	1.00 25.23	N
ATOM	256	CA	VAL	A	15	64.439	11.468	31.738	1.00 20.90	C
ATOM	257	C	VAL	A	15	65.654	10.713	32.378	1.00 17.26	C
ATOM	258	O	VAL	A	15	65.590	10.239	33.524	1.00 18.41	O
ATOM	259	CB	VAL	A	15	63.752	10.550	30.680	1.00 23.25	C
ATOM	260	CG1	VAL	A	15	63.330	9.253	31.310	1.00 15.71	C
ATOM	261	CG2	VAL	A	15	62.528	11.193	30.183	1.00 13.40	C
ATOM	262	N	GLY	A	16	66.784	10.642	31.665	1.00 20.39	N
ATOM	263	CA	GLY	A	16	67.941	9.904	32.186	1.00 19.54	C
ATOM	264	C	GLY	A	16	68.522	10.432	33.492	1.00 29.29	C
ATOM	265	O	GLY	A	16	68.896	9.659	34.434	1.00 16.91	O
ATOM	266	N	SER	A	17	68.642	11.755	33.499	1.00 12.53	N
ATOM	267	CA	SER	A	17	69.154	12.460	34.650	1.00 21.93	C
ATOM	268	C	SER	A	17	68.209	12.214	35.818	1.00 13.35	C
ATOM	269	O	SER	A	17	68.677	11.957	36.915	1.00 24.19	O
ATOM	270	CB	SER	A	17	69.378	13.942	34.333	1.00 15.52	C
ATOM	271	OG	SER	A	17	68.153	14.619	34.372	1.00 22.95	O
ATOM	272	N	ALA	A	18	66.896	12.143	35.590	1.00 17.52	N
ATOM	273	CA	ALA	A	18	65.991	11.828	36.729	1.00 13.14	C
ATOM	274	C	ALA	A	18	66.220	10.393	37.307	1.00 19.29	C
ATOM	275	O	ALA	A	18	66.149	10.150	38.522	1.00 16.94	O
ATOM	276	CB	ALA	A	18	64.460	12.046	36.334	1.00 14.33	C
ATOM	277	N	ILE	A	19	66.484	9.432	36.430	1.00 20.80	N
ATOM	278	CA	ILE	A	19	66.705	8.078	36.900	1.00 18.08	C
ATOM	279	C	ILE	A	19	67.975	8.090	37.730	1.00 16.09	C

ATOM	280	O	ILE	A	19	68.018	7.530	38.820	1.00	20.73	O
ATOM	281	CB	ILE	A	19	66.804	7.079	35.710	1.00	17.58	C
ATOM	282	CG1	ILE	A	19	65.444	6.812	35.162	1.00	10.09	C
ATOM	283	CG2	ILE	A	19	67.309	5.666	36.133	1.00	21.60	C
ATOM	284	CD1	ILE	A	19	65.528	6.361	33.741	1.00	19.05	C
ATOM	285	N	ARG	A	20	68.984	8.771	37.198	1.00	18.13	N
ATOM	286	CA	ARG	A	20	70.286	8.897	37.836	1.00	20.25	C
ATOM	287	C	ARG	A	20	70.231	9.491	39.242	1.00	30.62	C
ATOM	288	O	ARG	A	20	70.957	9.091	40.129	1.00	33.00	O
ATOM	289	CB	ARG	A	20	71.201	9.743	36.957	1.00	11.71	C
ATOM	290	CG	ARG	A	20	72.610	9.781	37.449	1.00	23.79	C
ATOM	291	CD	ARG	A	20	72.881	11.107	38.060	1.00	36.76	C
ATOM	292	NE	ARG	A	20	74.297	11.443	38.062	1.00	48.34	N
ATOM	293	CZ	ARG	A	20	74.990	11.841	36.988	1.00	100.00	C
ATOM	294	NH1	ARG	A	20	74.393	11.931	35.808	1.00	100.00	N
ATOM	295	NH2	ARG	A	20	76.289	12.139	37.076	1.00	100.00	N
ATOM	296	N	ARG	A	21	69.368	10.461	39.439	1.00	22.10	N
ATOM	297	CA	ARG	A	21	69.216	11.052	40.750	1.00	17.45	C
ATOM	298	C	ARG	A	21	68.721	10.007	41.730	1.00	26.71	C
ATOM	299	O	ARG	A	21	69.147	10.001	42.885	1.00	30.27	O
ATOM	300	CB	ARG	A	21	68.142	12.144	40.708	1.00	17.93	C
ATOM	301	CG	ARG	A	21	68.682	13.522	40.321	1.00	27.57	C
ATOM	302	CD	ARG	A	21	67.586	14.599	40.130	1.00	23.02	C
ATOM	303	NE	ARG	A	21	67.619	15.000	38.743	1.00	55.12	N
ATOM	304	CZ	ARG	A	21	66.538	15.103	37.995	1.00	10.55	C
ATOM	305	NH1	ARG	A	21	65.348	14.974	38.552	1.00	29.80	N
ATOM	306	NH2	ARG	A	21	66.665	15.435	36.715	1.00	61.45	N
ATOM	307	N	GLN	A	22	67.713	9.223	41.345	1.00	27.48	N
ATOM	308	CA	GLN	A	22	67.167	8.257	42.313	1.00	24.79	C
ATOM	309	C	GLN	A	22	68.137	7.127	42.547	1.00	31.37	C
ATOM	310	O	GLN	A	22	68.394	6.724	43.685	1.00	27.47	O
ATOM	311	CB	GLN	A	22	65.818	7.706	41.894	1.00	17.11	C
ATOM	312	CG	GLN	A	22	64.921	8.745	41.243	1.00	66.14	C
ATOM	313	CD	GLN	A	22	63.425	8.456	41.397	1.00	41.27	C
ATOM	314	OE1	GLN	A	22	63.002	7.329	41.762	1.00	29.34	O
ATOM	315	NE2	GLN	A	22	62.610	9.464	41.046	1.00	20.12	N
ATOM	316	N	LEU	A	23	68.697	6.652	41.448	1.00	27.99	N
ATOM	317	CA	LEU	A	23	69.649	5.575	41.500	1.00	24.48	C
ATOM	318	C	LEU	A	23	70.828	5.971	42.334	1.00	28.87	C
ATOM	319	O	LEU	A	23	71.288	5.218	43.165	1.00	30.79	O
ATOM	320	CB	LEU	A	23	70.036	5.107	40.089	1.00	22.72	C
ATOM	321	CG	LEU	A	23	68.966	4.072	39.658	1.00	26.16	C
ATOM	322	CD1	LEU	A	23	69.271	3.083	38.481	1.00	24.80	C
ATOM	323	CD2	LEU	A	23	68.427	3.284	40.835	1.00	22.91	C
ATOM	324	N	GLU	A	24	71.279	7.192	42.153	1.00	28.77	N

ATOM	325	CA	GLU	A	24	72.419	7.675	42.909	1.00	33.79	C
ATOM	326	C	GLU	A	24	72.363	7.388	44.412	1.00	35.94	C
ATOM	327	O	GLU	A	24	73.381	7.140	45.031	1.00	39.07	O
ATOM	328	CB	GLU	A	24	72.647	9.165	42.653	1.00	36.21	C
ATOM	329	CG	GLU	A	24	74.068	9.482	42.243	1.00	42.54	C
ATOM	330	CD	GLU	A	24	74.158	10.689	41.333	1.00	89.51	C
ATOM	331	OE1	GLU	A	24	73.386	11.663	41.549	1.00	43.21	O
ATOM	332	OE2	GLU	A	24	74.994	10.646	40.398	1.00	66.28	O
ATOM	333	N	GLN	A	25	71.182	7.422	45.000	1.00	45.70	N
ATOM	334	CA	GLN	A	25	71.039	7.152	46.432	1.00	47.57	C
ATOM	335	C	GLN	A	25	70.887	5.669	46.740	1.00	67.34	C
ATOM	336	O	GLN	A	25	70.285	5.286	47.726	1.00	74.06	O
ATOM	337	CB	GLN	A	25	69.783	7.842	46.905	1.00	51.85	C
ATOM	338	CG	GLN	A	25	69.500	9.084	46.109	1.00	44.91	C
ATOM	339	CD	GLN	A	25	68.419	9.913	46.742	1.00	100.00	C
ATOM	340	OE1	GLN	A	25	68.271	9.947	47.972	1.00	100.00	O
ATOM	341	NE2	GLN	A	25	67.624	10.602	45.911	1.00	100.00	N
ATOM	342	N	ARG	A	26	71.322	4.831	45.825	1.00	75.37	N
ATOM	343	CA	ARG	A	26	71.182	3.407	46.026	1.00	74.87	C
ATOM	344	C	ARG	A	26	72.568	2.791	46.147	1.00	74.08	C
ATOM	345	O	ARG	A	26	73.440	2.997	45.289	1.00	77.00	O
ATOM	346	CB	ARG	A	26	70.390	2.790	44.885	1.00	52.44	C
ATOM	347	CG	ARG	A	26	68.916	2.927	45.070	1.00	43.51	C
ATOM	348	CD	ARG	A	26	68.428	1.752	45.864	1.00	40.70	C
ATOM	349	NE	ARG	A	26	67.200	1.176	45.338	1.00	42.33	N
ATOM	350	CZ	ARG	A	26	67.126	0.508	44.196	1.00	32.07	C
ATOM	351	NH1	ARG	A	26	68.215	0.324	43.486	1.00	44.02	N
ATOM	352	NH2	ARG	A	26	65.968	0.017	43.771	1.00	77.32	N
ATOM	353	N	GLY	A	27	72.778	2.114	47.266	1.00	46.30	N
ATOM	354	CA	GLY	A	27	74.060	1.531	47.549	1.00	46.82	C
ATOM	355	C	GLY	A	27	74.140	0.165	46.923	1.00	55.45	C
ATOM	356	O	GLY	A	27	75.204	-0.453	46.877	1.00	64.43	O
ATOM	357	N	ASP	A	28	73.017	-0.315	46.428	1.00	40.98	N
ATOM	358	CA	ASP	A	28	73.016	-1.647	45.861	1.00	40.35	C
ATOM	359	C	ASP	A	28	73.266	-1.536	44.400	1.00	39.55	C
ATOM	360	O	ASP	A	28	73.109	-2.518	43.654	1.00	48.80	O
ATOM	361	CB	ASP	A	28	71.680	-2.335	46.127	1.00	47.80	C
ATOM	362	CG	ASP	A	28	70.503	-1.373	46.064	1.00	35.34	C
ATOM	363	OD1	ASP	A	28	70.705	-0.140	46.095	1.00	39.23	O
ATOM	364	OD2	ASP	A	28	69.383	-1.870	45.872	1.00	69.86	O
ATOM	365	N	VAL	A	29	73.651	-0.329	43.996	1.00	31.03	N
ATOM	366	CA	VAL	A	29	73.881	-0.050	42.591	1.00	28.44	C
ATOM	367	C	VAL	A	29	75.166	0.676	42.281	1.00	28.00	C
ATOM	368	O	VAL	A	29	75.505	1.699	42.892	1.00	34.83	O
ATOM	369	CB	VAL	A	29	72.696	0.760	42.000	1.00	30.68	C

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ATOM    370  CG1 VAL A  29      72.935   1.088  40.549  1.00 23.65           C
ATOM    371  CG2 VAL A  29      71.416  -0.028  42.156  1.00 27.95           C
ATOM    372  N   GLU A  30      75.824   0.219  41.230  1.00 30.76           N
ATOM    373  CA  GLU A  30      76.995   0.924  40.736  1.00 28.38           C
ATOM    374  C   GLU A  30      76.678   1.471  39.332  1.00 31.03           C
ATOM    375  O   GLU A  30      76.368   0.720  38.397  1.00 26.64           O
ATOM    376  CB  GLU A  30      78.199   0.006  40.722  1.00 31.84           C
ATOM    377  CG  GLU A  30      79.355   0.539  41.533  1.00 89.26           C
ATOM    378  CD  GLU A  30      80.667   0.264  40.858  1.00100.00           C
ATOM    379  OE1 GLU A  30      81.082  -0.922  40.872  1.00 88.94           O
ATOM    380  OE2 GLU A  30      81.202   1.206  40.219  1.00100.00           O
ATOM    381  N   LEU A  31      76.665   2.789  39.207  1.00 22.24           N
ATOM    382  CA  LEU A  31      76.269   3.391  37.945  1.00 29.37           C
ATOM    383  C   LEU A  31      77.404   3.507  36.941  1.00 25.79           C
ATOM    384  O   LEU A  31      78.485   3.969  37.256  1.00 29.41           O
ATOM    385  CB  LEU A  31      75.632   4.760  38.191  1.00 30.20           C
ATOM    386  CG  LEU A  31      74.329   4.763  38.994  1.00 29.37           C
ATOM    387  CD1 LEU A  31      73.841   6.143  39.240  1.00 23.43           C
ATOM    388  CD2 LEU A  31      73.275   3.962  38.281  1.00 23.04           C
ATOM    389  N   VAL A  32      77.146   3.100  35.711  1.00 21.94           N
ATOM    390  CA  VAL A  32      78.143   3.265  34.685  1.00 25.48           C
ATOM    391  C   VAL A  32      77.535   4.242  33.669  1.00 38.76           C
ATOM    392  O   VAL A  32      76.429   3.999  33.180  1.00 29.70           O
ATOM    393  CB  VAL A  32      78.517   1.902  34.055  1.00 34.25           C
ATOM    394  CG1 VAL A  32      79.587   2.079  32.970  1.00 30.56           C
ATOM    395  CG2 VAL A  32      79.003   0.950  35.139  1.00 25.27           C
ATOM    396  N   LEU A  33      78.219   5.375  33.457  1.00 30.19           N
ATOM    397  CA  LEU A  33      77.732   6.463  32.621  1.00 22.71           C
ATOM    398  C   LEU A  33      78.727   6.979  31.645  1.00 29.55           C
ATOM    399  O   LEU A  33      79.896   7.152  31.988  1.00 30.09           O
ATOM    400  CB  LEU A  33      77.423   7.635  33.514  1.00 19.75           C
ATOM    401  CG  LEU A  33      76.729   7.200  34.779  1.00 19.38           C
ATOM    402  CD1 LEU A  33      76.814   8.344  35.762  1.00 27.24           C
ATOM    403  CD2 LEU A  33      75.271   6.913  34.444  1.00 22.07           C
ATOM    404  N   ARG A  34      78.239   7.421  30.496  1.00 15.09           N
ATOM    405  CA  ARG A  34      79.154   8.008  29.541  1.00 26.04           C
ATOM    406  C   ARG A  34      78.469   9.173  28.916  1.00 36.57           C
ATOM    407  O   ARG A  34      77.288   9.130  28.651  1.00 38.59           O
ATOM    408  CB  ARG A  34      79.486   7.048  28.398  1.00 22.89           C
ATOM    409  CG  ARG A  34      80.579   6.081  28.706  1.00 23.29           C
ATOM    410  CD  ARG A  34      81.370   6.575  29.860  1.00 52.06           C
ATOM    411  NE  ARG A  34      81.783   5.458  30.711  1.00 80.25           N
ATOM    412  CZ  ARG A  34      82.646   4.530  30.323  1.00 41.94           C
ATOM    413  NH1 ARG A  34      83.173   4.596  29.104  1.00 53.02           N
ATOM    414  NH2 ARG A  34      82.983   3.547  31.148  1.00 25.56           N
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ATOM	415	N	THR	A	35	79.248	10.156	28.539	1.00 31.58	N
ATOM	416	CA	THR	A	35	78.703	11.282	27.833	1.00 29.33	C
ATOM	417	C	THR	A	35	78.719	10.951	26.340	1.00 32.53	C
ATOM	418	O	THR	A	35	79.350	9.944	25.962	1.00 28.08	O
ATOM	419	CB	THR	A	35	79.527	12.527	28.145	1.00 37.49	C
ATOM	420	OG1	THR	A	35	80.844	12.429	27.560	1.00 31.91	O
ATOM	421	CG2	THR	A	35	79.627	12.642	29.651	1.00 19.38	C
ATOM	422	N	ARG	A	36	78.032	11.780	25.529	1.00 30.02	N
ATOM	423	CA	ARG	A	36	78.002	11.639	24.056	1.00 29.37	C
ATOM	424	C	ARG	A	36	79.406	11.765	23.503	1.00 31.46	C
ATOM	425	O	ARG	A	36	79.772	11.012	22.591	1.00 36.56	O
ATOM	426	CB	ARG	A	36	77.054	12.650	23.354	1.00 37.34	C
ATOM	427	CG	ARG	A	36	76.937	12.465	21.846	-99.00 49.47	C
ATOM	428	CD	ARG	A	36	76.020	13.515	21.232	-99.00 63.09	C
ATOM	429	NE	ARG	A	36	75.528	13.124	19.915	-99.00 75.23	N
ATOM	430	CZ	ARG	A	36	74.381	13.549	19.391	-99.00 91.44	C
ATOM	431	NH1	ARG	A	36	73.605	14.375	20.079	-99.00 79.32	N
ATOM	432	NH2	ARG	A	36	74.009	13.144	18.185	-99.00 78.73	N
ATOM	433	N	ASP	A	37	80.217	12.677	24.063	1.00 41.30	N
ATOM	434	CA	ASP	A	37	81.606	12.710	23.601	1.00 44.91	C
ATOM	435	C	ASP	A	37	82.410	11.481	24.043	1.00 24.99	C
ATOM	436	O	ASP	A	37	83.211	10.978	23.261	1.00 42.22	O
ATOM	437	CB	ASP	A	37	82.347	14.048	23.718	-99.00 47.07	C
ATOM	438	CG	ASP	A	37	81.881	14.887	24.876	-99.00 62.99	C
ATOM	439	OD1	ASP	A	37	80.679	14.839	25.204	-99.00 64.45	O
ATOM	440	OD2	ASP	A	37	82.711	15.638	25.429	-99.00 69.84	O
ATOM	441	N	GLU	A	38	82.129	10.950	25.235	1.00 19.39	N
ATOM	442	CA	GLU	A	38	82.790	9.717	25.682	1.00 27.84	C
ATOM	443	C	GLU	A	38	82.203	8.527	24.901	1.00 37.14	C
ATOM	444	O	GLU	A	38	82.873	7.511	24.699	1.00 35.04	O
ATOM	445	CB	GLU	A	38	82.691	9.435	27.207	1.00 25.18	C
ATOM	446	CG	GLU	A	38	83.116	10.549	28.183	1.00 37.45	C
ATOM	447	CD	GLU	A	38	82.807	10.212	29.655	1.00 21.13	C
ATOM	448	OE1	GLU	A	38	81.623	9.997	30.014	1.00 55.97	O
ATOM	449	OE2	GLU	A	38	83.757	9.978	30.419	1.00 98.78	O
ATOM	450	N	LEU	A	39	80.948	8.610	24.478	1.00 25.52	N
ATOM	451	CA	LEU	A	39	80.440	7.483	23.739	1.00 18.17	C
ATOM	452	C	LEU	A	39	79.291	7.764	22.825	1.00 20.34	C
ATOM	453	O	LEU	A	39	78.152	7.810	23.259	1.00 26.35	O
ATOM	454	CB	LEU	A	39	80.123	6.313	24.657	1.00 14.56	C
ATOM	455	CG	LEU	A	39	79.410	5.075	24.058	1.00 19.52	C
ATOM	456	CD1	LEU	A	39	80.205	4.392	22.994	1.00 18.84	C
ATOM	457	CD2	LEU	A	39	78.890	4.051	25.084	1.00 17.41	C
ATOM	458	N	ASN	A	40	79.598	7.880	21.543	1.00 16.73	N
ATOM	459	CA	ASN	A	40	78.548	7.971	20.540	1.00 21.55	C

ATOM	460	C	ASN	A	40	77.798	6.649	20.308	1.00	24.53	C
ATOM	461	O	ASN	A	40	78.328	5.720	19.688	1.00	19.96	O
ATOM	462	CB	ASN	A	40	79.130	8.367	19.216	1.00	18.45	C
ATOM	463	CG	ASN	A	40	78.054	8.727	18.225	1.00	42.19	C
ATOM	464	OD1	ASN	A	40	78.327	9.093	17.080	1.00	38.89	O
ATOM	465	ND2	ASN	A	40	76.827	8.730	18.697	1.00	23.71	N
ATOM	466	N	LEU	A	41	76.543	6.622	20.754	1.00	21.08	N
ATOM	467	CA	LEU	A	41	75.649	5.465	20.650	1.00	15.03	C
ATOM	468	C	LEU	A	41	75.225	5.068	19.213	1.00	18.22	C
ATOM	469	O	LEU	A	41	74.681	3.971	18.980	1.00	15.72	O
ATOM	470	CB	LEU	A	41	74.426	5.705	21.532	1.00	15.85	C
ATOM	471	CG	LEU	A	41	74.822	6.029	22.974	1.00	21.90	C
ATOM	472	CD1	LEU	A	41	73.604	6.413	23.749	1.00	20.59	C
ATOM	473	CD2	LEU	A	41	75.481	4.796	23.609	1.00	17.97	C
ATOM	474	N	LEU	A	42	75.542	5.916	18.238	1.00	12.45	N
ATOM	475	CA	LEU	A	42	75.256	5.607	16.831	1.00	15.99	C
ATOM	476	C	LEU	A	42	76.290	4.680	16.280	1.00	26.18	C
ATOM	477	O	LEU	A	42	76.066	4.039	15.257	1.00	22.41	O
ATOM	478	CB	LEU	A	42	75.282	6.873	15.984	1.00	17.85	C
ATOM	479	CG	LEU	A	42	74.180	7.854	16.399	1.00	30.70	C
ATOM	480	CD1	LEU	A	42	74.318	9.184	15.704	1.00	24.31	C
ATOM	481	CD2	LEU	A	42	72.764	7.241	16.208	1.00	31.13	C
ATOM	482	N	ASP	A	43	77.462	4.705	16.911	1.00	26.87	N
ATOM	483	CA	ASP	A	43	78.579	3.875	16.486	1.00	19.29	C
ATOM	484	C	ASP	A	43	78.583	2.519	17.163	1.00	13.33	C
ATOM	485	O	ASP	A	43	79.051	2.348	18.297	1.00	18.75	O
ATOM	486	CB	ASP	A	43	79.870	4.580	16.776	1.00	31.06	C
ATOM	487	CG	ASP	A	43	81.083	3.758	16.380	1.00	30.68	C
ATOM	488	OD1	ASP	A	43	80.971	2.551	16.082	1.00	32.36	O
ATOM	489	OD2	ASP	A	43	82.187	4.308	16.499	1.00	37.83	O
ATOM	490	N	SER	A	44	78.139	1.544	16.377	1.00	16.89	N
ATOM	491	CA	SER	A	44	77.978	0.173	16.789	1.00	17.67	C
ATOM	492	C	SER	A	44	79.237	-0.463	17.392	1.00	20.40	C
ATOM	493	O	SER	A	44	79.206	-1.126	18.444	1.00	26.27	O
ATOM	494	CB	SER	A	44	77.504	-0.617	15.581	1.00	13.85	C
ATOM	495	OG	SER	A	44	76.800	-1.740	16.063	1.00	43.83	O
ATOM	496	N	ARG	A	45	80.335	-0.301	16.682	1.00	15.63	N
ATOM	497	CA	ARG	A	45	81.616	-0.788	17.154	1.00	19.94	C
ATOM	498	C	ARG	A	45	81.910	-0.225	18.521	1.00	29.48	C
ATOM	499	O	ARG	A	45	82.244	-0.937	19.457	1.00	27.65	O
ATOM	500	CB	ARG	A	45	82.684	-0.261	16.203	1.00	27.46	C
ATOM	501	CG	ARG	A	45	83.463	-1.338	15.495	1.00	92.03	C
ATOM	502	CD	ARG	A	45	84.854	-1.418	16.077	1.00	100.00	C
ATOM	503	NE	ARG	A	45	85.636	-2.533	15.527	1.00	100.00	N
ATOM	504	CZ	ARG	A	45	86.092	-3.570	16.236	1.00	100.00	C

ATOM 505 NH1 ARG A 45 85.791 -3.695 17.547 1.00100.00 N
ATOM 506 NH2 ARG A 45 86.773 -4.544 15.642 1.00100.00 N
ATOM 507 N ALA A 46 81.772 1.090 18.629 1.00 31.04 N
ATOM 508 CA ALA A 46 82.045 1.743 19.881 1.00 24.72 C
ATOM 509 C ALA A 46 81.111 1.176 20.899 1.00 17.73 C
ATOM 510 O ALA A 46 81.512 0.825 22.027 1.00 22.73 O
ATOM 511 CB ALA A 46 81.839 3.221 19.751 1.00 27.16 C
ATOM 512 N VAL A 47 79.835 1.119 20.531 1.00 17.54 N
ATOM 513 CA VAL A 47 78.878 0.608 21.508 1.00 21.41 C
ATOM 514 C VAL A 47 79.262 -0.812 21.914 1.00 30.25 C
ATOM 515 O VAL A 47 79.192 -1.202 23.097 1.00 15.85 O
ATOM 516 CB VAL A 47 77.470 0.668 20.989 1.00 18.59 C
ATOM 517 CG1 VAL A 47 76.503 0.042 22.012 1.00 16.88 C
ATOM 518 CG2 VAL A 47 77.115 2.096 20.756 1.00 16.28 C
ATOM 519 N HIS A 48 79.692 -1.585 20.920 1.00 21.00 N
ATOM 520 CA HIS A 48 80.028 -2.969 21.192 1.00 20.17 C
ATOM 521 C HIS A 48 81.268 -3.079 22.117 1.00 32.98 C
ATOM 522 O HIS A 48 81.289 -3.850 23.102 1.00 28.20 O
ATOM 523 CB HIS A 48 80.063 -3.801 19.855 1.00 14.93 C
ATOM 524 CG HIS A 48 78.686 -4.172 19.338 1.00 26.67 C
ATOM 525 ND1 HIS A 48 78.085 -5.394 19.600 1.00 28.83 N
ATOM 526 CD2 HIS A 48 77.758 -3.448 18.659 1.00 25.56 C
ATOM 527 CE1 HIS A 48 76.887 -5.430 19.043 1.00 20.08 C
ATOM 528 NE2 HIS A 48 76.660 -4.260 18.475 1.00 25.22 N
ATOM 529 N ASP A 49 82.217 -2.170 21.902 1.00 22.62 N
ATOM 530 CA ASP A 49 83.455 -2.169 22.674 1.00 24.23 C
ATOM 531 C ASP A 49 83.171 -1.899 24.122 1.00 38.72 C
ATOM 532 O ASP A 49 83.708 -2.551 25.027 1.00 35.44 O
ATOM 533 CB ASP A 49 84.396 -1.112 22.127 1.00 30.29 C
ATOM 534 CG ASP A 49 84.991 -1.503 20.775 1.00 52.45 C
ATOM 535 OD1 ASP A 49 85.007 -2.726 20.449 1.00 42.67 O
ATOM 536 OD2 ASP A 49 85.416 -0.587 20.029 1.00 73.76 O
ATOM 537 N PHE A 50 82.294 -0.929 24.324 1.00 32.19 N
ATOM 538 CA PHE A 50 81.902 -0.550 25.649 1.00 29.76 C
ATOM 539 C PHE A 50 81.299 -1.765 26.359 1.00 30.31 C
ATOM 540 O PHE A 50 81.715 -2.124 27.449 1.00 29.22 O
ATOM 541 CB PHE A 50 80.892 0.610 25.576 1.00 23.82 C
ATOM 542 CG PHE A 50 80.137 0.843 26.859 1.00 19.13 C
ATOM 543 CD1 PHE A 50 80.740 1.515 27.931 1.00 20.14 C
ATOM 544 CD2 PHE A 50 78.835 0.360 27.018 1.00 13.99 C
ATOM 545 CE1 PHE A 50 80.034 1.742 29.129 1.00 25.81 C
ATOM 546 CE2 PHE A 50 78.114 0.553 28.212 1.00 22.84 C
ATOM 547 CZ PHE A 50 78.698 1.276 29.259 1.00 23.40 C
ATOM 548 N PHE A 51 80.280 -2.367 25.768 1.00 21.75 N
ATOM 549 CA PHE A 51 79.655 -3.451 26.457 1.00 22.61 C

ATOM	550	C	PHE	A	51	80.646	-4.603	26.612	1.00	34.01	C
ATOM	551	O	PHE	A	51	80.550	-5.401	27.590	1.00	25.28	O
ATOM	552	CB	PHE	A	51	78.389	-3.898	25.751	1.00	22.63	C
ATOM	553	CG	PHE	A	51	77.158	-3.140	26.170	1.00	27.58	C
ATOM	554	CD1	PHE	A	51	76.426	-3.525	27.280	1.00	21.78	C
ATOM	555	CD2	PHE	A	51	76.663	-2.100	25.380	1.00	19.55	C
ATOM	556	CE1	PHE	A	51	75.267	-2.796	27.662	1.00	28.34	C
ATOM	557	CE2	PHE	A	51	75.492	-1.403	25.734	1.00	14.47	C
ATOM	558	CZ	PHE	A	51	74.797	-1.744	26.878	1.00	14.55	C
ATOM	559	N	ALA	A	52	81.576	-4.706	25.659	1.00	26.43	N
ATOM	560	CA	ALA	A	52	82.587	-5.793	25.714	1.00	29.44	C
ATOM	561	C	ALA	A	52	83.687	-5.560	26.768	1.00	43.76	C
ATOM	562	O	ALA	A	52	84.502	-6.446	27.022	1.00	40.33	O
ATOM	563	CB	ALA	A	52	83.228	-6.049	24.344	1.00	24.25	C
ATOM	564	N	SER	A	53	83.702	-4.382	27.385	1.00	31.96	N
ATOM	565	CA	SER	A	53	84.705	-4.090	28.377	1.00	21.06	C
ATOM	566	C	SER	A	53	84.196	-3.625	29.709	1.00	26.41	C
ATOM	567	O	SER	A	53	84.985	-3.492	30.611	1.00	36.12	O
ATOM	568	CB	SER	A	53	85.709	-3.088	27.843	1.00	14.22	C
ATOM	569	OG	SER	A	53	85.140	-1.807	27.790	1.00	56.90	O
ATOM	570	N	GLU	A	54	82.892	-3.431	29.874	1.00	22.38	N
ATOM	571	CA	GLU	A	54	82.380	-2.893	31.139	1.00	17.27	C
ATOM	572	C	GLU	A	54	81.584	-3.735	32.118	1.00	26.32	C
ATOM	573	O	GLU	A	54	81.229	-3.281	33.191	1.00	37.43	O
ATOM	574	CB	GLU	A	54	81.677	-1.563	30.906	1.00	27.30	C
ATOM	575	CG	GLU	A	54	82.573	-0.543	30.262	1.00	44.77	C
ATOM	576	CD	GLU	A	54	83.669	-0.142	31.194	1.00	86.31	C
ATOM	577	OE1	GLU	A	54	83.392	-0.232	32.428	1.00	50.11	O
ATOM	578	OE2	GLU	A	54	84.785	0.198	30.692	1.00	50.99	O
ATOM	579	N	ARG	A	55	81.268	-4.971	31.804	1.00	29.63	N
ATOM	580	CA	ARG	A	55	80.636	-5.748	32.854	1.00	33.32	C
ATOM	581	C	ARG	A	55	79.347	-5.149	33.378	1.00	38.45	C
ATOM	582	O	ARG	A	55	79.214	-4.897	34.576	1.00	40.18	O
ATOM	583	CB	ARG	A	55	81.621	-5.875	34.045	1.00	57.61	C
ATOM	584	CG	ARG	A	55	82.666	-7.028	33.960	1.00	100.00	C
ATOM	585	CD	ARG	A	55	82.805	-7.805	35.305	1.00	100.00	C
ATOM	586	NE	ARG	A	55	82.838	-9.270	35.146	1.00	100.00	N
ATOM	587	CZ	ARG	A	55	83.206	-10.129	36.102	1.00	100.00	C
ATOM	588	NH1	ARG	A	55	83.583	-9.681	37.301	1.00	100.00	N
ATOM	589	NH2	ARG	A	55	83.208	-11.440	35.855	1.00	100.00	N
ATOM	590	N	ILE	A	56	78.367	-5.029	32.491	1.00	42.25	N
ATOM	591	CA	ILE	A	56	77.064	-4.434	32.794	1.00	25.49	C
ATOM	592	C	ILE	A	56	75.982	-5.474	33.244	1.00	20.18	C
ATOM	593	O	ILE	A	56	75.897	-6.579	32.704	1.00	24.74	O
ATOM	594	CB	ILE	A	56	76.672	-3.512	31.531	1.00	26.89	C

ATOM	595	CG1	ILE	A	56	77.643	-2.301	31.442	1.00 18.30	C
ATOM	596	CG2	ILE	A	56	75.214	-3.016	31.549	1.00 19.84	C
ATOM	597	CD1	ILE	A	56	77.998	-1.936	30.026	1.00 60.42	C
ATOM	598	N	ASP	A	57	75.166	-5.133	34.237	1.00 16.84	N
ATOM	599	CA	ASP	A	57	74.040	-5.999	34.630	1.00 16.33	C
ATOM	600	C	ASP	A	57	72.676	-5.451	34.123	1.00 28.40	C
ATOM	601	O	ASP	A	57	71.836	-6.198	33.657	1.00 25.50	O
ATOM	602	CB	ASP	A	57	74.009	-6.194	36.164	1.00 16.94	C
ATOM	603	CG	ASP	A	57	75.369	-6.720	36.703	1.00 34.27	C
ATOM	604	OD1	ASP	A	57	75.875	-7.729	36.141	1.00 31.76	O
ATOM	605	OD2	ASP	A	57	76.040	-6.007	37.499	1.00 28.36	O
ATOM	606	N	GLN	A	58	72.443	-4.152	34.220	1.00 28.91	N
ATOM	607	CA	GLN	A	58	71.183	-3.590	33.755	1.00 25.68	C
ATOM	608	C	GLN	A	58	71.425	-2.364	32.881	1.00 23.21	C
ATOM	609	O	GLN	A	58	72.403	-1.620	33.067	1.00 18.16	O
ATOM	610	CB	GLN	A	58	70.342	-3.151	34.946	1.00 33.14	C
ATOM	611	CG	GLN	A	58	69.798	-4.241	35.807	1.00 30.00	C
ATOM	612	CD	GLN	A	58	69.226	-3.712	37.105	1.00 27.18	C
ATOM	613	OE1	GLN	A	58	68.722	-2.601	37.161	1.00 31.20	O
ATOM	614	NE2	GLN	A	58	69.455	-4.436	38.186	1.00 16.89	N
ATOM	615	N	VAL	A	59	70.496	-2.138	31.961	1.00 18.35	N
ATOM	616	CA	VAL	A	59	70.562	-0.998	31.045	1.00 15.59	C
ATOM	617	C	VAL	A	59	69.238	-0.240	31.039	1.00 26.28	C
ATOM	618	O	VAL	A	59	68.178	-0.820	30.762	1.00 19.51	O
ATOM	619	CB	VAL	A	59	70.707	-1.456	29.601	1.00 15.32	C
ATOM	620	CG1	VAL	A	59	70.477	-0.274	28.649	1.00 11.93	C
ATOM	621	CG2	VAL	A	59	72.080	-2.111	29.364	1.00 15.83	C
ATOM	622	N	TYR	A	60	69.306	1.064	31.293	1.00 21.71	N
ATOM	623	CA	TYR	A	60	68.113	1.927	31.197	1.00 21.40	C
ATOM	624	C	TYR	A	60	68.289	2.756	29.928	1.00 18.69	C
ATOM	625	O	TYR	A	60	69.250	3.532	29.796	1.00 15.51	O
ATOM	626	CB	TYR	A	60	68.021	2.817	32.413	1.00 17.24	C
ATOM	627	CG	TYR	A	60	67.493	2.131	33.658	1.00 19.71	C
ATOM	628	CD1	TYR	A	60	68.345	1.583	34.586	1.00 21.14	C
ATOM	629	CD2	TYR	A	60	66.154	2.223	33.991	1.00 20.16	C
ATOM	630	CE1	TYR	A	60	67.835	1.080	35.794	1.00 19.11	C
ATOM	631	CE2	TYR	A	60	65.648	1.698	35.163	1.00 10.77	C
ATOM	632	CZ	TYR	A	60	66.476	1.094	36.054	1.00 20.07	C
ATOM	633	OH	TYR	A	60	65.921	0.585	37.248	1.00 16.04	O
ATOM	634	N	LEU	A	61	67.491	2.452	28.916	1.00 17.46	N
ATOM	635	CA	LEU	A	61	67.685	3.053	27.585	1.00 20.17	C
ATOM	636	C	LEU	A	61	67.003	4.412	27.409	1.00 23.36	C
ATOM	637	O	LEU	A	61	65.925	4.526	26.799	1.00 14.86	O
ATOM	638	CB	LEU	A	61	67.267	2.060	26.485	1.00 14.78	C
ATOM	639	CG	LEU	A	61	68.117	2.142	25.208	1.00 15.52	C

ATOM	640	CD1	LEU	A	61	67.815	1.010	24.109	1.00	7.75	C
ATOM	641	CD2	LEU	A	61	68.087	3.541	24.580	1.00	15.20	C
ATOM	642	N	ALA	A	62	67.656	5.434	27.956	1.00	20.35	N
ATOM	643	CA	ALA	A	62	67.120	6.784	27.963	1.00	18.55	C
ATOM	644	C	ALA	A	62	67.779	7.739	26.949	1.00	18.57	C
ATOM	645	O	ALA	A	62	67.455	8.924	26.920	1.00	24.31	O
ATOM	646	CB	ALA	A	62	67.071	7.377	29.439	1.00	11.69	C
ATOM	647	N	ALA	A	63	68.681	7.231	26.101	1.00	14.09	N
ATOM	648	CA	ALA	A	63	69.249	8.095	25.052	1.00	12.84	C
ATOM	649	C	ALA	A	63	68.310	8.005	23.877	1.00	27.00	C
ATOM	650	O	ALA	A	63	67.845	6.916	23.511	1.00	24.51	O
ATOM	651	CB	ALA	A	63	70.665	7.660	24.634	1.00	4.89	C
ATOM	652	N	ALA	A	64	68.076	9.148	23.262	1.00	21.05	N
ATOM	653	CA	ALA	A	64	67.202	9.286	22.086	1.00	13.50	C
ATOM	654	C	ALA	A	64	67.435	10.664	21.416	1.00	28.08	C
ATOM	655	O	ALA	A	64	67.987	11.600	22.021	1.00	26.63	O
ATOM	656	CB	ALA	A	64	65.642	9.171	22.518	1.00	7.63	C
ATOM	657	N	LYS	A	65	66.953	10.781	20.182	1.00	23.98	N
ATOM	658	CA	LYS	A	65	66.966	12.012	19.409	1.00	20.47	C
ATOM	659	C	LYS	A	65	65.488	12.443	19.551	1.00	24.37	C
ATOM	660	O	LYS	A	65	64.594	11.807	18.976	1.00	20.29	O
ATOM	661	CB	LYS	A	65	67.317	11.658	17.951	1.00	25.59	C
ATOM	662	CG	LYS	A	65	66.808	12.630	16.923	1.00	27.54	C
ATOM	663	CD	LYS	A	65	67.518	13.926	17.169	1.00	21.08	C
ATOM	664	CE	LYS	A	65	67.316	14.905	16.029	1.00	55.15	C
ATOM	665	NZ	LYS	A	65	67.876	16.263	16.392	1.00	81.63	N
ATOM	666	N	VAL	A	66	65.228	13.362	20.485	1.00	22.47	N
ATOM	667	CA	VAL	A	66	63.873	13.850	20.755	1.00	18.99	C
ATOM	668	C	VAL	A	66	63.711	15.343	20.394	1.00	31.44	C
ATOM	669	O	VAL	A	66	64.665	16.107	20.460	1.00	34.61	O
ATOM	670	CB	VAL	A	66	63.440	13.623	22.204	1.00	16.66	C
ATOM	671	CG1	VAL	A	66	64.269	12.623	22.869	1.00	15.01	C
ATOM	672	CG2	VAL	A	66	63.379	14.904	22.950	1.00	19.21	C
ATOM	673	N	GLY	A	67	62.514	15.755	19.994	1.00	18.03	N
ATOM	674	CA	GLY	A	67	62.298	17.149	19.614	1.00	14.90	C
ATOM	675	C	GLY	A	67	60.792	17.518	19.585	1.00	32.35	C
ATOM	676	O	GLY	A	67	59.922	16.666	19.888	1.00	18.88	O
ATOM	677	N	GLY	A	68	60.503	18.787	19.256	1.00	23.21	N
ATOM	678	CA	GLY	A	68	59.132	19.288	19.183	1.00	23.83	C
ATOM	679	C	GLY	A	68	58.540	19.137	17.771	1.00	19.31	C
ATOM	680	O	GLY	A	68	59.165	18.550	16.870	1.00	30.64	O
ATOM	681	N	ILE	A	69	57.343	19.684	17.588	1.00	15.20	N
ATOM	682	CA	ILE	A	69	56.595	19.632	16.317	1.00	16.80	C
ATOM	683	C	ILE	A	69	57.387	20.153	15.112	1.00	19.33	C
ATOM	684	O	ILE	A	69	57.425	19.519	14.061	1.00	14.66	O

ATOM	685	CB	ILE	A	69	55.257	20.432	16.480	1.00	30.11	C
ATOM	686	CG1	ILE	A	69	54.271	19.683	17.385	1.00	24.27	C
ATOM	687	CG2	ILE	A	69	54.610	20.749	15.181	1.00	47.53	C
ATOM	688	CD1	ILE	A	69	53.259	20.608	18.056	1.00	85.71	C
ATOM	689	N	VAL	A	70	58.010	21.327	15.269	1.00	23.03	N
ATOM	690	CA	VAL	A	70	58.797	21.913	14.183	1.00	19.34	C
ATOM	691	C	VAL	A	70	59.983	21.011	13.840	1.00	24.42	C
ATOM	692	O	VAL	A	70	60.335	20.829	12.662	1.00	24.14	O
ATOM	693	CB	VAL	A	70	59.304	23.404	14.467	1.00	21.37	C
ATOM	694	CG1	VAL	A	70	60.137	23.907	13.281	1.00	17.79	C
ATOM	695	CG2	VAL	A	70	58.136	24.410	14.678	1.00	15.74	C
ATOM	696	N	ALA	A	71	60.621	20.450	14.861	1.00	19.68	N
ATOM	697	CA	ALA	A	71	61.782	19.617	14.572	1.00	16.57	C
ATOM	698	C	ALA	A	71	61.427	18.289	13.910	1.00	23.36	C
ATOM	699	O	ALA	A	71	61.980	17.923	12.849	1.00	21.84	O
ATOM	700	CB	ALA	A	71	62.685	19.439	15.805	1.00	9.36	C
ATOM	701	N	ASN	A	72	60.463	17.598	14.511	1.00	16.80	N
ATOM	702	CA	ASN	A	72	59.998	16.357	13.923	1.00	18.84	C
ATOM	703	C	ASN	A	72	59.608	16.539	12.440	1.00	23.87	C
ATOM	704	O	ASN	A	72	59.919	15.696	11.593	1.00	21.52	O
ATOM	705	CB	ASN	A	72	58.835	15.806	14.738	1.00	8.60	C
ATOM	706	CG	ASN	A	72	59.309	15.013	15.911	1.00	23.75	C
ATOM	707	OD1	ASN	A	72	59.558	13.809	15.810	1.00	23.98	O
ATOM	708	ND2	ASN	A	72	59.572	15.701	16.996	1.00	9.96	N
ATOM	709	N	ASN	A	73	58.931	17.647	12.138	1.00	23.07	N
ATOM	710	CA	ASN	A	73	58.521	17.971	10.761	1.00	26.05	C
ATOM	711	C	ASN	A	73	59.665	18.454	9.817	1.00	26.95	C
ATOM	712	O	ASN	A	73	59.613	18.276	8.569	1.00	22.13	O
ATOM	713	CB	ASN	A	73	57.383	19.001	10.800	1.00	14.86	C
ATOM	714	CG	ASN	A	73	56.015	18.349	10.987	1.00	19.88	C
ATOM	715	OD1	ASN	A	73	55.620	17.468	10.217	1.00	27.02	O
ATOM	716	ND2	ASN	A	73	55.322	18.732	12.051	1.00	20.78	N
ATOM	717	N	THR	A	74	60.710	19.029	10.419	1.00	18.69	N
ATOM	718	CA	THR	A	74	61.845	19.540	9.657	1.00	10.07	C
ATOM	719	C	THR	A	74	62.968	18.548	9.375	1.00	21.00	C
ATOM	720	O	THR	A	74	63.537	18.561	8.289	1.00	11.75	O
ATOM	721	CB	THR	A	74	62.411	20.746	10.306	1.00	29.10	C
ATOM	722	OG1	THR	A	74	61.370	21.714	10.457	1.00	23.24	O
ATOM	723	CG2	THR	A	74	63.541	21.299	9.452	1.00	21.63	C
ATOM	724	N	TYR	A	75	63.230	17.636	10.310	1.00	17.10	N
ATOM	725	CA	TYR	A	75	64.267	16.620	10.112	1.00	9.07	C
ATOM	726	C	TYR	A	75	63.733	15.203	10.318	1.00	6.17	C
ATOM	727	O	TYR	A	75	64.143	14.542	11.267	1.00	15.58	O
ATOM	728	CB	TYR	A	75	65.302	16.825	11.188	1.00	11.89	C
ATOM	729	CG	TYR	A	75	65.779	18.234	11.252	1.00	27.12	C

ATOM	730	CD1	TYR	A	75	66.712	18.696	10.321	1.00	28.46	C
ATOM	731	CD2	TYR	A	75	65.234	19.151	12.173	1.00	24.83	C
ATOM	732	CE1	TYR	A	75	67.117	20.045	10.305	1.00	28.34	C
ATOM	733	CE2	TYR	A	75	65.652	20.523	12.180	1.00	21.00	C
ATOM	734	CZ	TYR	A	75	66.593	20.940	11.234	1.00	45.42	C
ATOM	735	OH	TYR	A	75	67.066	22.230	11.215	1.00	35.37	O
ATOM	736	N	PRO	A	76	62.759	14.775	9.532	1.00	13.30	N
ATOM	737	CA	PRO	A	76	62.185	13.438	9.742	1.00	14.64	C
ATOM	738	C	PRO	A	76	63.209	12.264	9.618	1.00	14.40	C
ATOM	739	O	PRO	A	76	63.157	11.335	10.409	1.00	20.54	O
ATOM	740	CB	PRO	A	76	61.055	13.366	8.709	1.00	7.83	C
ATOM	741	CG	PRO	A	76	61.447	14.388	7.617	1.00	12.61	C
ATOM	742	CD	PRO	A	76	62.068	15.504	8.455	1.00	11.18	C
ATOM	743	N	ALA	A	77	64.163	12.339	8.681	1.00	15.25	N
ATOM	744	CA	ALA	A	77	65.206	11.312	8.538	1.00	6.79	C
ATOM	745	C	ALA	A	77	66.053	11.166	9.820	1.00	17.22	C
ATOM	746	O	ALA	A	77	66.306	10.069	10.292	1.00	18.74	O
ATOM	747	CB	ALA	A	77	66.097	11.601	7.330	1.00	9.04	C
ATOM	748	N	ASP	A	78	66.466	12.267	10.424	1.00	10.92	N
ATOM	749	CA	ASP	A	78	67.256	12.191	11.659	1.00	11.87	C
ATOM	750	C	ASP	A	78	66.572	11.486	12.827	1.00	16.09	C
ATOM	751	O	ASP	A	78	67.212	10.741	13.601	1.00	18.07	O
ATOM	752	CB	ASP	A	78	67.578	13.609	12.088	1.00	19.16	C
ATOM	753	CG	ASP	A	78	68.424	14.325	11.068	1.00	26.82	C
ATOM	754	OD1	ASP	A	78	68.836	13.694	10.044	1.00	33.93	O
ATOM	755	OD2	ASP	A	78	68.673	15.514	11.316	1.00	32.06	O
ATOM	756	N	PHE	A	79	65.279	11.771	12.975	1.00	14.70	N
ATOM	757	CA	PHE	A	79	64.471	11.192	14.044	1.00	20.69	C
ATOM	758	C	PHE	A	79	64.224	9.707	13.876	1.00	20.22	C
ATOM	759	O	PHE	A	79	64.269	8.987	14.862	1.00	22.37	O
ATOM	760	CB	PHE	A	79	63.144	11.933	14.219	1.00	27.38	C
ATOM	761	CG	PHE	A	79	63.264	13.218	14.990	1.00	28.59	C
ATOM	762	CD1	PHE	A	79	63.137	13.230	16.386	1.00	27.49	C
ATOM	763	CD2	PHE	A	79	63.509	14.415	14.325	1.00	28.20	C
ATOM	764	CE1	PHE	A	79	63.281	14.413	17.109	1.00	21.76	C
ATOM	765	CE2	PHE	A	79	63.625	15.593	15.037	1.00	31.48	C
ATOM	766	CZ	PHE	A	79	63.509	15.582	16.439	1.00	26.31	C
ATOM	767	N	ILE	A	80	63.942	9.249	12.650	1.00	10.79	N
ATOM	768	CA	ILE	A	80	63.828	7.795	12.410	1.00	18.12	C
ATOM	769	C	ILE	A	80	65.197	7.052	12.432	1.00	10.97	C
ATOM	770	O	ILE	A	80	65.406	6.090	13.195	1.00	8.92	O
ATOM	771	CB	ILE	A	80	62.944	7.408	11.148	1.00	17.41	C
ATOM	772	CG1	ILE	A	80	62.651	5.886	11.105	1.00	10.16	C
ATOM	773	CG2	ILE	A	80	63.583	7.888	9.901	1.00	17.46	C
ATOM	774	CD1	ILE	A	80	61.722	5.410	9.980	1.00	7.30	C

ATOM	775	N	TYR A	81	66.151	7.539	11.658	1.00	11.18	N
ATOM	776	CA	TYR A	81	67.488	6.902	11.630	1.00	15.06	C
ATOM	777	C	TYR A	81	68.237	6.782	12.959	1.00	16.83	C
ATOM	778	O	TYR A	81	68.714	5.702	13.383	1.00	16.74	O
ATOM	779	CB	TYR A	81	68.384	7.599	10.616	1.00	9.43	C
ATOM	780	CG	TYR A	81	69.749	6.966	10.541	1.00	22.54	C
ATOM	781	CD1	TYR A	81	69.963	5.824	9.747	1.00	22.37	C
ATOM	782	CD2	TYR A	81	70.818	7.466	11.299	1.00	18.07	C
ATOM	783	CE1	TYR A	81	71.202	5.163	9.746	1.00	15.02	C
ATOM	784	CE2	TYR A	81	72.080	6.893	11.201	1.00	17.37	C
ATOM	785	CZ	TYR A	81	72.255	5.698	10.472	1.00	24.27	C
ATOM	786	OH	TYR A	81	73.491	5.063	10.409	1.00	19.57	O
ATOM	787	N	GLN A	82	68.385	7.918	13.612	1.00	11.39	N
ATOM	788	CA	GLN A	82	69.193	7.930	14.810	1.00	12.23	C
ATOM	789	C	GLN A	82	68.544	7.089	15.834	1.00	14.18	C
ATOM	790	O	GLN A	82	69.180	6.415	16.631	1.00	11.35	O
ATOM	791	CB	GLN A	82	69.280	9.354	15.291	1.00	18.73	C
ATOM	792	CG	GLN A	82	69.986	10.209	14.250	1.00	13.54	C
ATOM	793	CD	GLN A	82	70.285	11.617	14.736	1.00	26.00	C
ATOM	794	OE1	GLN A	82	70.410	11.850	15.927	1.00	22.99	O
ATOM	795	NE2	GLN A	82	70.404	12.561	13.808	1.00	16.59	N
ATOM	796	N	ASN A	83	67.235	7.181	15.869	1.00	11.35	N
ATOM	797	CA	ASN A	83	66.549	6.408	16.860	1.00	13.71	C
ATOM	798	C	ASN A	83	66.623	4.902	16.557	1.00	21.43	C
ATOM	799	O	ASN A	83	66.831	4.101	17.463	1.00	12.10	O
ATOM	800	CB	ASN A	83	65.132	6.945	17.074	1.00	13.51	C
ATOM	801	CG	ASN A	83	65.131	8.245	17.871	1.00	28.91	C
ATOM	802	OD1	ASN A	83	65.628	8.263	18.990	1.00	22.28	O
ATOM	803	ND2	ASN A	83	64.756	9.354	17.237	1.00	20.17	N
ATOM	804	N	MET A	84	66.592	4.517	15.290	1.00	15.63	N
ATOM	805	CA	MET A	84	66.704	3.101	15.007	1.00	15.66	C
ATOM	806	C	MET A	84	68.054	2.588	15.348	1.00	14.66	C
ATOM	807	O	MET A	84	68.148	1.514	15.902	1.00	11.45	O
ATOM	808	CB	MET A	84	66.418	2.815	13.563	1.00	17.59	C
ATOM	809	CG	MET A	84	64.911	2.894	13.220	1.00	14.40	C
ATOM	810	SD	MET A	84	64.638	2.811	11.387	1.00	15.99	S
ATOM	811	CE	MET A	84	65.164	1.105	10.952	1.00	8.90	C
ATOM	812	N	MET A	85	69.098	3.338	15.024	1.00	11.20	N
ATOM	813	CA	MET A	85	70.468	2.879	15.321	1.00	11.67	C
ATOM	814	C	MET A	85	70.779	2.831	16.774	1.00	13.04	C
ATOM	815	O	MET A	85	71.359	1.893	17.265	1.00	15.26	O
ATOM	816	CB	MET A	85	71.525	3.798	14.693	1.00	15.07	C
ATOM	817	CG	MET A	85	71.530	3.726	13.173	1.00	32.01	C
ATOM	818	SD	MET A	85	71.918	2.027	12.487	1.00	37.79	S
ATOM	819	CE	MET A	85	73.379	1.801	13.320	1.00	15.94	C

ATOM	820	N	ILE	A	86	70.471	3.892	17.481	1.00	13.92	N
ATOM	821	CA	ILE	A	86	70.760	3.893	18.912	1.00	12.58	C
ATOM	822	C	ILE	A	86	70.159	2.662	19.591	1.00	21.61	C
ATOM	823	O	ILE	A	86	70.813	1.981	20.362	1.00	18.68	O
ATOM	824	CB	ILE	A	86	70.225	5.189	19.606	1.00	11.84	C
ATOM	825	CG1	ILE	A	86	70.978	6.429	19.119	1.00	19.78	C
ATOM	826	CG2	ILE	A	86	70.435	5.132	21.112	1.00	6.59	C
ATOM	827	CD1	ILE	A	86	70.505	7.694	19.772	1.00	20.37	C
ATOM	828	N	GLU	A	87	68.893	2.383	19.316	1.00	18.78	N
ATOM	829	CA	GLU	A	87	68.263	1.237	19.930	1.00	14.00	C
ATOM	830	C	GLU	A	87	68.797	-0.116	19.454	1.00	15.93	C
ATOM	831	O	GLU	A	87	69.017	-0.991	20.268	1.00	11.04	O
ATOM	832	CB	GLU	A	87	66.734	1.324	19.900	1.00	14.89	C
ATOM	833	CG	GLU	A	87	66.085	1.327	18.538	1.00	28.96	C
ATOM	834	CD	GLU	A	87	64.635	1.922	18.544	1.00	11.12	C
ATOM	835	OE1	GLU	A	87	64.307	2.801	19.376	1.00	25.46	O
ATOM	836	OE2	GLU	A	87	63.845	1.547	17.663	1.00	29.87	O
ATOM	837	N	SER	A	88	69.054	-0.259	18.155	1.00	16.18	N
ATOM	838	CA	SER	A	88	69.650	-1.482	17.569	1.00	19.52	C
ATOM	839	C	SER	A	88	71.029	-1.792	18.160	1.00	22.54	C
ATOM	840	O	SER	A	88	71.313	-2.929	18.592	1.00	13.80	O
ATOM	841	CB	SER	A	88	69.815	-1.326	16.023	1.00	14.61	C
ATOM	842	OG	SER	A	88	68.551	-1.201	15.355	1.00	15.41	O
ATOM	843	N	ASN	A	89	71.884	-0.773	18.143	1.00	22.63	N
ATOM	844	CA	ASN	A	89	73.227	-0.869	18.693	1.00	27.23	C
ATOM	845	C	ASN	A	89	73.195	-1.363	20.134	1.00	21.34	C
ATOM	846	O	ASN	A	89	73.795	-2.384	20.476	1.00	23.68	O
ATOM	847	CB	ASN	A	89	73.980	0.487	18.597	1.00	13.71	C
ATOM	848	CG	ASN	A	89	74.440	0.825	17.168	1.00	20.40	C
ATOM	849	OD1	ASN	A	89	74.305	-0.006	16.255	1.00	14.93	O
ATOM	850	ND2	ASN	A	89	74.937	2.067	16.960	1.00	13.32	N
ATOM	851	N	ILE	A	90	72.488	-0.646	20.979	1.00	16.55	N
ATOM	852	CA	ILE	A	90	72.437	-1.014	22.398	1.00	21.51	C
ATOM	853	C	ILE	A	90	71.876	-2.421	22.729	1.00	26.50	C
ATOM	854	O	ILE	A	90	72.384	-3.159	23.590	1.00	19.71	O
ATOM	855	CB	ILE	A	90	71.670	0.070	23.233	1.00	13.32	C
ATOM	856	CG1	ILE	A	90	72.539	1.299	23.401	1.00	11.05	C
ATOM	857	CG2	ILE	A	90	71.371	-0.445	24.637	1.00	7.54	C
ATOM	858	CD1	ILE	A	90	71.749	2.597	23.668	1.00	20.71	C
ATOM	859	N	ILE	A	91	70.755	-2.733	22.114	1.00	14.98	N
ATOM	860	CA	ILE	A	91	70.047	-3.953	22.442	1.00	21.33	C
ATOM	861	C	ILE	A	91	70.927	-5.098	21.994	1.00	26.27	C
ATOM	862	O	ILE	A	91	71.211	-6.011	22.751	1.00	26.56	O
ATOM	863	CB	ILE	A	91	68.556	-3.930	21.814	1.00	20.39	C
ATOM	864	CG1	ILE	A	91	67.692	-2.886	22.552	1.00	13.51	C

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ATOM    865  CG2 ILE A  91      67.841  -5.316  21.845  1.00 11.31           C
ATOM    866  CD1 ILE A  91      66.320  -2.648  21.907  1.00 16.23           C
ATOM    867  N   HIS A  92      71.446  -4.983  20.785  1.00 24.12           N
ATOM    868  CA  HIS A  92      72.293  -6.015  20.243  1.00 26.71           C
ATOM    869  C   HIS A  92      73.609  -6.251  21.071  1.00 29.30           C
ATOM    870  O   HIS A  92      73.983  -7.366  21.443  1.00 18.58           O
ATOM    871  CB  HIS A  92      72.561  -5.682  18.775  1.00 22.23           C
ATOM    872  CG  HIS A  92      73.366  -6.720  18.077  1.00 26.32           C
ATOM    873  ND1 HIS A  92      72.798  -7.711  17.307  1.00 27.19           N
ATOM    874  CD2 HIS A  92      74.699  -6.978  18.106  1.00 21.95           C
ATOM    875  CE1 HIS A  92      73.755  -8.487  16.826  1.00 23.66           C
ATOM    876  NE2 HIS A  92      74.918  -8.062  17.296  1.00 17.36           N
ATOM    877  N   ALA A  93      74.328  -5.187  21.333  1.00 15.66           N
ATOM    878  CA  ALA A  93      75.530  -5.301  22.110  1.00 11.88           C
ATOM    879  C   ALA A  93      75.222  -5.900  23.512  1.00 28.78           C
ATOM    880  O   ALA A  93      75.912  -6.790  24.037  1.00 25.23           O
ATOM    881  CB  ALA A  93      76.139  -3.959  22.221  1.00  6.30           C
ATOM    882  N   ALA A  94      74.142  -5.442  24.113  1.00 18.82           N
ATOM    883  CA  ALA A  94      73.777  -5.971  25.399  1.00 15.61           C
ATOM    884  C   ALA A  94      73.593  -7.503  25.301  1.00 28.39           C
ATOM    885  O   ALA A  94      74.133  -8.263  26.099  1.00 21.67           O
ATOM    886  CB  ALA A  94      72.449  -5.279  25.911  1.00 18.46           C
ATOM    887  N   HIS A  95      72.814  -7.966  24.329  1.00 26.35           N
ATOM    888  CA  HIS A  95      72.551  -9.396  24.271  1.00 24.89           C
ATOM    889  C   HIS A  95      73.845 -10.176  24.140  1.00 22.81           C
ATOM    890  O   HIS A  95      74.077 -11.136  24.865  1.00 21.44           O
ATOM    891  CB  HIS A  95      71.571  -9.778  23.129  1.00 22.39           C
ATOM    892  CG  HIS A  95      71.554 -11.250  22.831  1.00 28.73           C
ATOM    893  ND1 HIS A  95      70.979 -12.182  23.682  1.00 22.83           N
ATOM    894  CD2 HIS A  95      72.159 -11.964  21.845  1.00 25.22           C
ATOM    895  CE1 HIS A  95      71.171 -13.397  23.196  1.00 22.72           C
ATOM    896  NE2 HIS A  95      71.911 -13.296  22.101  1.00 24.80           N
ATOM    897  N   GLN A  96      74.709  -9.658  23.281  1.00 19.97           N
ATOM    898  CA  GLN A  96      75.960 -10.299  22.917  1.00 22.27           C
ATOM    899  C   GLN A  96      76.877 -10.353  24.086  1.00 26.58           C
ATOM    900  O   GLN A  96      77.836 -11.093  24.088  1.00 24.17           O
ATOM    901  CB  GLN A  96      76.642  -9.492  21.818  1.00 23.38           C
ATOM    902  CG  GLN A  96      77.043 -10.299  20.596  1.00 61.06           C
ATOM    903  CD  GLN A  96      78.033  -9.557  19.675  1.00 75.83           C
ATOM    904  OE1 GLN A  96      78.999  -8.941  20.131  1.00 56.89           O
ATOM    905  NE2 GLN A  96      77.815  -9.668  18.366  1.00100.00           N
ATOM    906  N   ASN A  97      76.652  -9.500  25.060  1.00 22.15           N
ATOM    907  CA  ASN A  97      77.537  -9.536  26.208  1.00 14.74           C
ATOM    908  C   ASN A  97      76.732 -10.022  27.387  1.00 29.78           C
ATOM    909  O   ASN A  97      77.049  -9.762  28.564  1.00 27.09           O
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ATOM    910  CB  ASN A  97      78.241  -8.201  26.462  1.00 12.93           C
ATOM    911  CG  ASN A  97      79.260  -7.897  25.407  1.00 24.91           C
ATOM    912  OD1 ASN A  97      80.331  -8.518  25.375  1.00 57.17           O
ATOM    913  ND2 ASN A  97      78.839  -7.135  24.392  1.00 34.88           N
ATOM    914  N   ASP A  98      75.666 -10.732  27.055  1.00 27.98           N
ATOM    915  CA  ASP A  98      74.907 -11.361  28.089  1.00 29.25           C
ATOM    916  C   ASP A  98      74.400 -10.379  29.164  1.00 37.53           C
ATOM    917  O   ASP A  98      74.505 -10.634  30.367  1.00 36.42           O
ATOM    918  CB  ASP A  98      75.791 -12.450  28.700  1.00 36.37           C
ATOM    919  CG  ASP A  98      75.016 -13.712  29.053  1.00 88.62           C
ATOM    920  OD1 ASP A  98      73.775 -13.749  28.877  1.00 82.53           O
ATOM    921  OD2 ASP A  98      75.656 -14.670  29.542  1.00100.00           O
ATOM    922  N   VAL A  99      73.879  -9.235  28.730  1.00 27.13           N
ATOM    923  CA  VAL A  99      73.157  -8.351  29.635  1.00 21.57           C
ATOM    924  C   VAL A  99      71.706  -8.868  29.530  1.00 16.15           C
ATOM    925  O   VAL A  99      71.159  -9.088  28.422  1.00 19.47           O
ATOM    926  CB  VAL A  99      73.264  -6.900  29.206  1.00 24.18           C
ATOM    927  CG1 VAL A  99      72.517  -6.015  30.198  1.00 14.58           C
ATOM    928  CG2 VAL A  99      74.720  -6.515  29.225  1.00 30.10           C
ATOM    929  N   ASN A 100      71.149  -9.262  30.662  1.00 17.39           N
ATOM    930  CA  ASN A 100      69.852  -9.925  30.613  1.00 25.77           C
ATOM    931  C   ASN A 100      68.648  -9.034  30.910  1.00 24.95           C
ATOM    932  O   ASN A 100      67.498  -9.377  30.582  1.00 20.88           O
ATOM    933  CB  ASN A 100      69.846 -11.157  31.527  1.00 14.98           C
ATOM    934  CG  ASN A 100      68.724 -12.112  31.180  1.00 20.38           C
ATOM    935  OD1 ASN A 100      68.737 -12.709  30.100  1.00 29.59           O
ATOM    936  ND2 ASN A 100      67.716 -12.240  32.076  1.00 16.35           N
ATOM    937  N   LYS A 101      68.941  -7.923  31.584  1.00 17.91           N
ATOM    938  CA  LYS A 101      67.970  -6.916  31.994  1.00 25.43           C
ATOM    939  C   LYS A 101      68.107  -5.510  31.323  1.00 25.29           C
ATOM    940  O   LYS A 101      69.151  -4.850  31.377  1.00 19.88           O
ATOM    941  CB  LYS A 101      67.996  -6.807  33.521  1.00 29.28           C
ATOM    942  CG  LYS A 101      67.464  -8.054  34.205  1.00  9.31           C
ATOM    943  CD  LYS A 101      67.218  -7.719  35.668  1.00 38.93           C
ATOM    944  CE  LYS A 101      66.206  -6.569  35.885  1.00 13.38           C
ATOM    945  NZ  LYS A 101      64.750  -7.006  35.825  1.00 15.26           N
ATOM    946  N   LEU A 102      67.013  -5.043  30.732  1.00 22.22           N
ATOM    947  CA  LEU A 102      67.003  -3.744  30.092  1.00 15.40           C
ATOM    948  C   LEU A 102      65.612  -3.115  30.156  1.00 18.55           C
ATOM    949  O   LEU A 102      64.590  -3.811  30.102  1.00 18.92           O
ATOM    950  CB  LEU A 102      67.465  -3.898  28.636  1.00 11.23           C
ATOM    951  CG  LEU A 102      67.553  -2.711  27.651  1.00 15.51           C
ATOM    952  CD1 LEU A 102      68.628  -2.985  26.559  1.00  9.65           C
ATOM    953  CD2 LEU A 102      66.162  -2.407  26.995  1.00 13.10           C
ATOM    954  N   LEU A 103      65.595  -1.798  30.318  1.00 17.05           N
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ATOM    955  CA  LEU A 103      64.356  -1.036  30.265  1.00 16.23           C
ATOM    956  C   LEU A 103      64.346  -0.072  29.046  1.00 19.65           C
ATOM    957  O   LEU A 103      65.215   0.789  28.875  1.00 19.68           O
ATOM    958  CB  LEU A 103      64.099  -0.289  31.562  1.00 12.28           C
ATOM    959  CG  LEU A 103      62.686   0.259  31.594  1.00 14.13           C
ATOM    960  CD1 LEU A 103      61.645  -0.822  31.902  1.00 10.31           C
ATOM    961  CD2 LEU A 103      62.646   1.360  32.601  1.00 12.30           C
ATOM    962  N   PHE A 104      63.417  -0.333  28.140  1.00 16.41           N
ATOM    963  CA  PHE A 104      63.215   0.486  26.956  1.00 18.32           C
ATOM    964  C   PHE A 104      62.126   1.546  27.249  1.00 21.85           C
ATOM    965  O   PHE A 104      61.168   1.271  27.992  1.00 18.36           O
ATOM    966  CB  PHE A 104      62.796  -0.386  25.793  1.00  9.86           C
ATOM    967  CG  PHE A 104      62.732   0.348  24.508  1.00 16.81           C
ATOM    968  CD1 PHE A 104      63.894   0.714  23.840  1.00 25.04           C
ATOM    969  CD2 PHE A 104      61.511   0.795  24.005  1.00 22.59           C
ATOM    970  CE1 PHE A 104      63.836   1.448  22.619  1.00 31.26           C
ATOM    971  CE2 PHE A 104      61.449   1.535  22.814  1.00 15.59           C
ATOM    972  CZ  PHE A 104      62.625   1.895  22.139  1.00 11.67           C
ATOM    973  N   LEU A 105      62.341   2.762  26.734  1.00 20.33           N
ATOM    974  CA  LEU A 105      61.416   3.897  26.904  1.00 18.10           C
ATOM    975  C   LEU A 105      60.711   4.237  25.634  1.00 17.04           C
ATOM    976  O   LEU A 105      61.315   4.680  24.665  1.00 18.83           O
ATOM    977  CB  LEU A 105      62.178   5.146  27.214  1.00 17.49           C
ATOM    978  CG  LEU A 105      62.434   5.544  28.644  1.00 27.17           C
ATOM    979  CD1 LEU A 105      62.630   4.349  29.574  1.00 19.16           C
ATOM    980  CD2 LEU A 105      63.688   6.347  28.529  1.00 23.59           C
ATOM    981  N   GLY A 106      59.407   4.153  25.652  1.00 20.66           N
ATOM    982  CA  GLY A 106      58.679   4.536  24.455  1.00 21.03           C
ATOM    983  C   GLY A 106      58.080   5.935  24.597  1.00 17.32           C
ATOM    984  O   GLY A 106      58.690   6.858  25.113  1.00 26.89           O
ATOM    985  N   SER A 107      56.831   6.047  24.219  1.00 22.05           N
ATOM    986  CA  SER A 107      56.177   7.317  24.288  1.00 22.12           C
ATOM    987  C   SER A 107      54.686   7.212  23.923  1.00 19.06           C
ATOM    988  O   SER A 107      54.314   6.545  22.963  1.00 27.42           O
ATOM    989  CB  SER A 107      56.882   8.232  23.300  1.00 20.99           C
ATOM    990  OG  SER A 107      55.947   9.133  22.776  1.00 42.85           O
ATOM    991  N   SER A 108      53.826   7.890  24.671  1.00 27.42           N
ATOM    992  CA  SER A 108      52.382   7.947  24.339  1.00 26.43           C
ATOM    993  C   SER A 108      52.144   8.259  22.842  1.00 30.97           C
ATOM    994  O   SER A 108      51.242   7.709  22.217  1.00 33.46           O
ATOM    995  CB  SER A 108      51.710   9.072  25.144  1.00 19.87           C
ATOM    996  OG  SER A 108      52.495  10.266  25.071  1.00 70.88           O
ATOM    997  N   CYS A 109      52.927   9.180  22.278  1.00 24.73           N
ATOM    998  CA  CYS A 109      52.728   9.549  20.880  1.00 25.61           C
ATOM    999  C   CYS A 109      52.970   8.482  19.815  1.00 21.29           C
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ATOM	1000	O	CYS A 109	52.967	8.737	18.623	1.00	31.31		O
ATOM	1001	CB	CYS A 109	53.369	10.899	20.544	1.00	39.55		C
ATOM	1002	SG	CYS A 109	55.153	11.077	20.847	1.00	49.24		S
ATOM	1003	N	ILE A 110	53.101	7.264	20.258	1.00	18.31		N
ATOM	1004	CA	ILE A 110	53.329	6.150	19.379	1.00	28.10		C
ATOM	1005	C	ILE A 110	51.977	5.489	19.082	1.00	15.38		C
ATOM	1006	O	ILE A 110	51.895	4.592	18.268	1.00	16.52		O
ATOM	1007	CB	ILE A 110	54.154	5.153	20.206	1.00	40.45		C
ATOM	1008	CG1	ILE A 110	55.604	5.510	20.136	1.00	39.02		C
ATOM	1009	CG2	ILE A 110	53.879	3.715	19.875	1.00	61.33		C
ATOM	1010	CD1	ILE A 110	56.429	4.338	20.549	1.00	82.74		C
ATOM	1011	N	TYR A 111	50.951	5.842	19.854	1.00	14.91		N
ATOM	1012	CA	TYR A 111	49.630	5.227	19.678	1.00	13.96		C
ATOM	1013	C	TYR A 111	48.956	5.831	18.459	1.00	20.40		C
ATOM	1014	O	TYR A 111	49.302	6.933	18.056	1.00	11.71		O
ATOM	1015	CB	TYR A 111	48.763	5.468	20.921	1.00	9.63		C
ATOM	1016	CG	TYR A 111	49.117	4.550	22.065	1.00	14.94		C
ATOM	1017	CD1	TYR A 111	48.985	3.159	21.938	1.00	9.73		C
ATOM	1018	CD2	TYR A 111	49.755	5.038	23.216	1.00	14.96		C
ATOM	1019	CE1	TYR A 111	49.344	2.273	23.014	1.00	6.53		C
ATOM	1020	CE2	TYR A 111	50.146	4.155	24.272	1.00	13.66		C
ATOM	1021	CZ	TYR A 111	49.873	2.787	24.171	1.00	17.86		C
ATOM	1022	OH	TYR A 111	50.266	1.927	25.157	1.00	11.37		O
ATOM	1023	N	PRO A 112	47.974	5.145	17.872	1.00	22.56		N
ATOM	1024	CA	PRO A 112	47.279	5.743	16.721	1.00	23.44		C
ATOM	1025	C	PRO A 112	46.589	7.111	16.988	1.00	17.82		C
ATOM	1026	O	PRO A 112	46.197	7.453	18.115	1.00	19.72		O
ATOM	1027	CB	PRO A 112	46.290	4.644	16.252	1.00	15.69		C
ATOM	1028	CG	PRO A 112	46.895	3.343	16.769	1.00	22.83		C
ATOM	1029	CD	PRO A 112	47.593	3.733	18.086	1.00	16.10		C
ATOM	1030	N	LYS A 113	46.418	7.866	15.915	1.00	19.48		N
ATOM	1031	CA	LYS A 113	45.793	9.167	15.994	1.00	23.50		C
ATOM	1032	C	LYS A 113	44.396	9.077	16.655	1.00	34.28		C
ATOM	1033	O	LYS A 113	44.046	9.887	17.524	1.00	46.14		O
ATOM	1034	CB	LYS A 113	45.675	9.735	14.593	1.00	30.04		C
ATOM	1035	CG	LYS A 113	46.219	11.124	14.477	1.00	43.78		C
ATOM	1036	CD	LYS A 113	45.381	11.941	13.515	1.00	100.00		C
ATOM	1037	CE	LYS A 113	44.361	12.836	14.250	1.00	100.00		C
ATOM	1038	NZ	LYS A 113	43.480	13.625	13.304	1.00	100.00		N
ATOM	1039	N	LEU A 114	43.591	8.103	16.250	1.00	26.33		N
ATOM	1040	CA	LEU A 114	42.267	7.957	16.833	1.00	20.65		C
ATOM	1041	C	LEU A 114	42.083	6.792	17.760	1.00	18.44		C
ATOM	1042	O	LEU A 114	41.002	6.278	17.918	1.00	34.04		O
ATOM	1043	CB	LEU A 114	41.194	8.002	15.780	1.00	24.37		C
ATOM	1044	CG	LEU A 114	41.587	9.122	14.830	1.00	40.86		C

ATOM	1045	CD1	LEU	A	114	40.991	8.797	13.504	1.00	49.29	C
ATOM	1046	CD2	LEU	A	114	41.139	10.512	15.300	1.00	26.85	C
ATOM	1047	N	ALA	A	115	43.103	6.473	18.527	1.00	29.00	N
ATOM	1048	CA	ALA	A	115	42.920	5.446	19.528	1.00	25.66	C
ATOM	1049	C	ALA	A	115	41.722	5.727	20.454	1.00	28.76	C
ATOM	1050	O	ALA	A	115	41.364	6.855	20.682	1.00	24.12	O
ATOM	1051	CB	ALA	A	115	44.177	5.272	20.326	1.00	16.86	C
ATOM	1052	N	LYS	A	116	41.137	4.675	20.998	1.00	30.21	N
ATOM	1053	CA	LYS	A	116	40.036	4.792	21.928	1.00	25.85	C
ATOM	1054	C	LYS	A	116	40.668	5.248	23.195	1.00	14.18	C
ATOM	1055	O	LYS	A	116	41.750	4.781	23.535	1.00	23.51	O
ATOM	1056	CB	LYS	A	116	39.369	3.415	22.116	1.00	22.05	C
ATOM	1057	CG	LYS	A	116	39.053	3.032	23.524	1.00	55.38	C
ATOM	1058	CD	LYS	A	116	37.963	1.955	23.549	1.00	100.00	C
ATOM	1059	CE	LYS	A	116	37.120	1.953	24.835	1.00	100.00	C
ATOM	1060	NZ	LYS	A	116	35.767	1.310	24.630	1.00	100.00	N
ATOM	1061	N	GLN	A	117	40.021	6.208	23.856	1.00	18.23	N
ATOM	1062	CA	GLN	A	117	40.456	6.757	25.180	1.00	21.01	C
ATOM	1063	C	GLN	A	117	39.695	6.178	26.383	1.00	30.96	C
ATOM	1064	O	GLN	A	117	38.483	6.009	26.345	1.00	27.66	O
ATOM	1065	CB	GLN	A	117	40.215	8.263	25.179	1.00	11.32	C
ATOM	1066	CG	GLN	A	117	40.849	8.912	23.948	1.00	12.12	C
ATOM	1067	CD	GLN	A	117	42.404	8.823	23.954	1.00	24.10	C
ATOM	1068	OE1	GLN	A	117	43.041	8.628	22.896	1.00	47.88	O
ATOM	1069	NE2	GLN	A	117	43.001	8.953	25.131	1.00	14.24	N
ATOM	1070	N	PRO	A	118	40.374	5.992	27.499	1.00	30.02	N
ATOM	1071	CA	PRO	A	118	41.826	6.194	27.655	1.00	26.44	C
ATOM	1072	C	PRO	A	118	42.450	5.050	26.899	1.00	24.37	C
ATOM	1073	O	PRO	A	118	41.792	4.027	26.726	1.00	25.34	O
ATOM	1074	CB	PRO	A	118	42.055	5.994	29.167	1.00	23.89	C
ATOM	1075	CG	PRO	A	118	40.847	5.240	29.654	1.00	23.20	C
ATOM	1076	CD	PRO	A	118	39.695	5.519	28.709	1.00	15.79	C
ATOM	1077	N	MET	A	119	43.684	5.228	26.432	1.00	16.00	N
ATOM	1078	CA	MET	A	119	44.372	4.215	25.644	1.00	10.80	C
ATOM	1079	C	MET	A	119	45.062	3.083	26.444	1.00	23.61	C
ATOM	1080	O	MET	A	119	46.013	3.281	27.209	1.00	18.02	O
ATOM	1081	CB	MET	A	119	45.384	4.894	24.791	1.00	13.52	C
ATOM	1082	CG	MET	A	119	44.801	6.014	23.989	1.00	18.52	C
ATOM	1083	SD	MET	A	119	46.157	7.054	23.271	1.00	26.27	S
ATOM	1084	CE	MET	A	119	46.264	6.524	21.845	1.00	33.79	C
ATOM	1085	N	ALA	A	120	44.559	1.875	26.271	1.00	26.64	N
ATOM	1086	CA	ALA	A	120	45.177	0.712	26.884	1.00	29.17	C
ATOM	1087	C	ALA	A	120	46.356	0.308	25.984	1.00	23.21	C
ATOM	1088	O	ALA	A	120	46.439	0.759	24.833	1.00	20.19	O
ATOM	1089	CB	ALA	A	120	44.169	-0.419	26.944	1.00	26.02	C

ATOM	1090	N	GLU A 121	47.238	-0.553	26.507	1.00	12.30		N
ATOM	1091	CA	GLU A 121	48.427	-1.009	25.788	1.00	9.45		C
ATOM	1092	C	GLU A 121	48.070	-1.697	24.450	1.00	11.68		C
ATOM	1093	O	GLU A 121	48.828	-1.670	23.450	1.00	14.84		O
ATOM	1094	CB	GLU A 121	49.321	-1.883	26.715	1.00	16.74		C
ATOM	1095	CG	GLU A 121	50.132	-1.122	27.763	1.00	18.14		C
ATOM	1096	CD	GLU A 121	49.458	-1.000	29.137	1.00	13.00		C
ATOM	1097	OE1	GLU A 121	48.252	-1.294	29.276	1.00	20.79		O
ATOM	1098	OE2	GLU A 121	50.123	-0.521	30.080	1.00	17.86		O
ATOM	1099	N	SER A 122	46.887	-2.273	24.409	1.00	11.79		N
ATOM	1100	CA	SER A 122	46.427	-2.977	23.218	1.00	12.16		C
ATOM	1101	C	SER A 122	46.030	-2.058	22.100	1.00	11.70		C
ATOM	1102	O	SER A 122	45.717	-2.529	21.010	1.00	13.91		O
ATOM	1103	CB	SER A 122	45.186	-3.781	23.568	1.00	21.50		C
ATOM	1104	OG	SER A 122	44.143	-2.908	23.976	1.00	28.52		O
ATOM	1105	N	GLU A 123	46.041	-0.754	22.341	1.00	14.65		N
ATOM	1106	CA	GLU A 123	45.783	0.202	21.243	1.00	17.15		C
ATOM	1107	C	GLU A 123	46.959	0.313	20.240	1.00	11.48		C
ATOM	1108	O	GLU A 123	46.821	0.844	19.141	1.00	11.19		O
ATOM	1109	CB	GLU A 123	45.481	1.600	21.805	1.00	21.66		C
ATOM	1110	CG	GLU A 123	44.127	1.694	22.523	1.00	24.68		C
ATOM	1111	CD	GLU A 123	42.984	1.374	21.585	1.00	35.56		C
ATOM	1112	OE1	GLU A 123	43.019	1.865	20.426	1.00	41.73		O
ATOM	1113	OE2	GLU A 123	42.158	0.497	21.940	1.00	100.00		O
ATOM	1114	N	LEU A 124	48.134	-0.185	20.618	1.00	14.02		N
ATOM	1115	CA	LEU A 124	49.296	-0.082	19.740	1.00	15.32		C
ATOM	1116	C	LEU A 124	49.082	-0.754	18.458	1.00	17.76		C
ATOM	1117	O	LEU A 124	48.752	-1.917	18.445	1.00	18.91		O
ATOM	1118	CB	LEU A 124	50.564	-0.680	20.362	1.00	18.07		C
ATOM	1119	CG	LEU A 124	51.922	-0.222	19.803	1.00	21.52		C
ATOM	1120	CD1	LEU A 124	52.080	1.258	20.117	1.00	20.35		C
ATOM	1121	CD2	LEU A 124	53.042	-0.919	20.550	1.00	14.07		C
ATOM	1122	N	LEU A 125	49.514	-0.071	17.409	1.00	18.44		N
ATOM	1123	CA	LEU A 125	49.445	-0.564	16.052	1.00	19.92		C
ATOM	1124	C	LEU A 125	48.034	-0.754	15.509	1.00	25.56		C
ATOM	1125	O	LEU A 125	47.854	-1.188	14.364	1.00	18.26		O
ATOM	1126	CB	LEU A 125	50.355	-1.800	15.840	1.00	20.79		C
ATOM	1127	CG	LEU A 125	51.890	-1.511	15.778	1.00	17.21		C
ATOM	1128	CD1	LEU A 125	52.744	-2.649	16.316	1.00	19.95		C
ATOM	1129	CD2	LEU A 125	52.334	-1.219	14.338	1.00	5.81		C
ATOM	1130	N	GLN A 126	47.027	-0.327	16.276	1.00	21.97		N
ATOM	1131	CA	GLN A 126	45.652	-0.504	15.790	1.00	19.97		C
ATOM	1132	C	GLN A 126	45.213	0.447	14.724	1.00	28.31		C
ATOM	1133	O	GLN A 126	44.076	0.391	14.293	1.00	47.49		O
ATOM	1134	CB	GLN A 126	44.652	-0.404	16.911	1.00	19.87		C

ATOM	1135	CG	GLN A 126	44.949	-1.312	18.048	1.00	18.39		C
ATOM	1136	CD	GLN A 126	44.319	-2.626	17.835	1.00	66.80		C
ATOM	1137	OE1	GLN A 126	44.064	-3.376	18.792	1.00	40.75		O
ATOM	1138	NE2	GLN A 126	44.015	-2.952	16.565	1.00	71.74		N
ATOM	1139	N	GLY A 127	46.080	1.330	14.270	1.00	28.29		N
ATOM	1140	CA	GLY A 127	45.627	2.260	13.252	1.00	23.31		C
ATOM	1141	C	GLY A 127	46.662	3.315	12.953	1.00	22.90		C
ATOM	1142	O	GLY A 127	47.755	3.254	13.474	1.00	25.30		O
ATOM	1143	N	THR A 128	46.311	4.219	12.046	1.00	19.51		N
ATOM	1144	CA	THR A 128	47.149	5.314	11.588	1.00	22.12		C
ATOM	1145	C	THR A 128	47.705	6.219	12.695	1.00	22.60		C
ATOM	1146	O	THR A 128	47.061	6.461	13.731	1.00	18.58		O
ATOM	1147	CB	THR A 128	46.392	6.182	10.544	1.00	35.98		C
ATOM	1148	OG1	THR A 128	46.533	5.594	9.239	1.00	58.05		O
ATOM	1149	CG2	THR A 128	46.942	7.639	10.542	1.00	43.41		C
ATOM	1150	N	LEU A 129	48.907	6.715	12.425	1.00	18.32		N
ATOM	1151	CA	LEU A 129	49.674	7.534	13.356	1.00	16.76		C
ATOM	1152	C	LEU A 129	49.504	8.959	12.967	1.00	4.89		C
ATOM	1153	O	LEU A 129	49.232	9.260	11.814	1.00	16.14		O
ATOM	1154	CB	LEU A 129	51.205	7.191	13.261	1.00	17.91		C
ATOM	1155	CG	LEU A 129	51.769	5.804	13.752	1.00	18.21		C
ATOM	1156	CD1	LEU A 129	53.132	5.379	13.193	1.00	12.12		C
ATOM	1157	CD2	LEU A 129	51.683	5.532	15.251	1.00	3.89		C
ATOM	1158	N	GLU A 130	49.816	9.827	13.917	1.00	10.23		N
ATOM	1159	CA	GLU A 130	49.912	11.268	13.691	1.00	13.22		C
ATOM	1160	C	GLU A 130	51.128	11.544	12.775	1.00	23.44		C
ATOM	1161	O	GLU A 130	52.249	11.162	13.090	1.00	21.23		O
ATOM	1162	CB	GLU A 130	50.150	11.979	15.035	1.00	18.48		C
ATOM	1163	CG	GLU A 130	50.754	13.376	14.886	1.00	77.44		C
ATOM	1164	CD	GLU A 130	49.833	14.328	14.121	1.00	100.00		C
ATOM	1165	OE1	GLU A 130	48.588	14.205	14.340	1.00	36.19		O
ATOM	1166	OE2	GLU A 130	50.347	15.161	13.295	1.00	21.03		O
ATOM	1167	N	PRO A 131	50.920	12.219	11.648	1.00	21.35		N
ATOM	1168	CA	PRO A 131	52.023	12.409	10.731	1.00	14.78		C
ATOM	1169	C	PRO A 131	53.201	13.132	11.265	1.00	14.98		C
ATOM	1170	O	PRO A 131	54.325	12.847	10.853	1.00	20.99		O
ATOM	1171	CB	PRO A 131	51.413	13.154	9.552	1.00	14.76		C
ATOM	1172	CG	PRO A 131	50.071	13.485	9.949	1.00	20.99		C
ATOM	1173	CD	PRO A 131	49.641	12.626	11.047	1.00	17.25		C
ATOM	1174	N	THR A 132	52.986	14.095	12.159	1.00	18.77		N
ATOM	1175	CA	THR A 132	54.131	14.838	12.689	1.00	16.44		C
ATOM	1176	C	THR A 132	55.102	13.951	13.408	1.00	21.91		C
ATOM	1177	O	THR A 132	56.317	14.088	13.234	1.00	24.17		O
ATOM	1178	CB	THR A 132	53.716	15.907	13.606	1.00	23.45		C
ATOM	1179	OG1	THR A 132	52.976	16.883	12.850	1.00	31.15		O

ATOM	1180	CG2	THR	A	132	54.969	16.519	14.341	1.00	9.28	C
ATOM	1181	N	ASN	A	133	54.551	12.970	14.122	1.00	28.59	N
ATOM	1182	CA	ASN	A	133	55.359	12.007	14.875	1.00	26.38	C
ATOM	1183	C	ASN	A	133	55.666	10.682	14.207	1.00	14.85	C
ATOM	1184	O	ASN	A	133	56.446	9.884	14.755	1.00	18.67	O
ATOM	1185	CB	ASN	A	133	54.661	11.699	16.168	1.00	23.70	C
ATOM	1186	CG	ASN	A	133	54.480	12.894	16.968	1.00	50.55	C
ATOM	1187	OD1	ASN	A	133	53.354	13.272	17.252	1.00	40.07	O
ATOM	1188	ND2	ASN	A	133	55.568	13.638	17.163	1.00	40.36	N
ATOM	1189	N	GLU	A	134	55.100	10.469	13.022	1.00	9.98	N
ATOM	1190	CA	GLU	A	134	55.237	9.210	12.365	1.00	9.66	C
ATOM	1191	C	GLU	A	134	56.648	8.530	12.274	1.00	13.86	C
ATOM	1192	O	GLU	A	134	56.814	7.388	12.706	1.00	22.89	O
ATOM	1193	CB	GLU	A	134	54.448	9.200	11.070	1.00	17.55	C
ATOM	1194	CG	GLU	A	134	54.750	7.930	10.227	1.00	20.89	C
ATOM	1195	CD	GLU	A	134	53.926	7.868	8.970	1.00	13.59	C
ATOM	1196	OE1	GLU	A	134	52.678	7.738	9.085	1.00	35.28	O
ATOM	1197	OE2	GLU	A	134	54.497	8.048	7.869	1.00	13.44	O
ATOM	1198	N	PRO	A	135	57.680	9.222	11.789	1.00	15.72	N
ATOM	1199	CA	PRO	A	135	59.014	8.600	11.699	1.00	18.91	C
ATOM	1200	C	PRO	A	135	59.544	8.174	13.073	1.00	18.68	C
ATOM	1201	O	PRO	A	135	60.072	7.069	13.271	1.00	15.69	O
ATOM	1202	CB	PRO	A	135	59.896	9.755	11.169	1.00	13.84	C
ATOM	1203	CG	PRO	A	135	59.036	10.514	10.350	1.00	9.78	C
ATOM	1204	CD	PRO	A	135	57.594	10.395	10.908	1.00	14.43	C
ATOM	1205	N	TYR	A	136	59.449	9.117	13.994	1.00	8.64	N
ATOM	1206	CA	TYR	A	136	59.873	8.915	15.324	1.00	13.27	C
ATOM	1207	C	TYR	A	136	59.056	7.728	15.907	1.00	16.84	C
ATOM	1208	O	TYR	A	136	59.578	6.903	16.658	1.00	12.90	O
ATOM	1209	CB	TYR	A	136	59.604	10.234	16.100	1.00	15.51	C
ATOM	1210	CG	TYR	A	136	59.912	10.168	17.614	1.00	18.26	C
ATOM	1211	CD1	TYR	A	136	61.200	10.062	18.072	1.00	20.53	C
ATOM	1212	CD2	TYR	A	136	58.904	10.150	18.568	1.00	17.38	C
ATOM	1213	CE1	TYR	A	136	61.484	9.959	19.440	1.00	30.44	C
ATOM	1214	CE2	TYR	A	136	59.184	10.084	19.953	1.00	9.85	C
ATOM	1215	CZ	TYR	A	136	60.476	9.949	20.377	1.00	20.65	C
ATOM	1216	OH	TYR	A	136	60.792	9.873	21.734	1.00	24.41	O
ATOM	1217	N	ALA	A	137	57.760	7.687	15.638	1.00	7.19	N
ATOM	1218	CA	ALA	A	137	56.923	6.633	16.227	1.00	12.68	C
ATOM	1219	C	ALA	A	137	57.345	5.265	15.737	1.00	15.21	C
ATOM	1220	O	ALA	A	137	57.425	4.272	16.488	1.00	14.58	O
ATOM	1221	CB	ALA	A	137	55.517	6.849	15.871	1.00	11.40	C
ATOM	1222	N	ILE	A	138	57.567	5.213	14.447	1.00	8.93	N
ATOM	1223	CA	ILE	A	138	57.954	3.971	13.831	1.00	11.77	C
ATOM	1224	C	ILE	A	138	59.246	3.494	14.492	1.00	16.20	C

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ATOM   1225  O   ILE A 138      59.307   2.377  14.970  1.00 13.79           O
ATOM   1226  CB  ILE A 138      58.064   4.172  12.316  1.00 17.85           C
ATOM   1227  CG1 ILE A 138      56.680   4.473  11.757  1.00 28.21           C
ATOM   1228  CG2 ILE A 138      58.674   2.986  11.602  1.00  9.81           C
ATOM   1229  CD1 ILE A 138      55.695   3.376  11.970  1.00 18.17           C
ATOM   1230  N   ALA A 139      60.243   4.361  14.625  1.00 11.54           N
ATOM   1231  CA  ALA A 139      61.494   3.937  15.288  1.00 13.22           C
ATOM   1232  C   ALA A 139      61.256   3.364  16.675  1.00 18.73           C
ATOM   1233  O   ALA A 139      61.791   2.318  17.031  1.00 20.44           O
ATOM   1234  CB  ALA A 139      62.434   5.073  15.390  1.00 13.62           C
ATOM   1235  N   LYS A 140      60.397   4.033  17.448  1.00 16.36           N
ATOM   1236  CA  LYS A 140      60.083   3.600  18.815  1.00 15.14           C
ATOM   1237  C   LYS A 140      59.392   2.262  18.824  1.00 15.18           C
ATOM   1238  O   LYS A 140      59.824   1.346  19.475  1.00 21.42           O
ATOM   1239  CB  LYS A 140      59.193   4.606  19.525  1.00 17.86           C
ATOM   1240  CG  LYS A 140      59.925   5.806  20.152  1.00 21.11           C
ATOM   1241  CD  LYS A 140      61.208   5.478  20.958  1.00 16.75           C
ATOM   1242  CE  LYS A 140      61.664   6.735  21.835  1.00 10.06           C
ATOM   1243  NZ  LYS A 140      62.688   6.496  22.921  1.00 14.40           N
ATOM   1244  N   ILE A 141      58.356   2.116  18.027  1.00 11.49           N
ATOM   1245  CA  ILE A 141      57.703   0.828  17.977  1.00 17.92           C
ATOM   1246  C   ILE A 141      58.729  -0.282  17.577  1.00 13.46           C
ATOM   1247  O   ILE A 141      58.730  -1.374  18.148  1.00 13.92           O
ATOM   1248  CB  ILE A 141      56.497   0.925  17.019  1.00 22.59           C
ATOM   1249  CG1 ILE A 141      55.466   1.906  17.557  1.00 17.61           C
ATOM   1250  CG2 ILE A 141      55.863  -0.411  16.700  1.00 10.49           C
ATOM   1251  CD1 ILE A 141      54.530   2.327  16.449  1.00 13.43           C
ATOM   1252  N   ALA A 142      59.637   0.028  16.650  1.00 10.29           N
ATOM   1253  CA  ALA A 142      60.657  -0.931  16.228  1.00  7.15           C
ATOM   1254  C   ALA A 142      61.456  -1.301  17.456  1.00 16.58           C
ATOM   1255  O   ALA A 142      61.839  -2.454  17.621  1.00 13.04           O
ATOM   1256  CB  ALA A 142      61.604  -0.288  15.130  1.00  4.44           C
ATOM   1257  N   GLY A 143      61.703  -0.307  16.316  1.00  9.56           N
ATOM   1258  CA  GLY A 143      62.448  -0.525  19.527  1.00  5.15           C
ATOM   1259  C   GLY A 143      61.770  -1.555  20.430  1.00 16.36           C
ATOM   1260  O   GLY A 143      62.392  -2.482  20.967  1.00 14.11           O
ATOM   1261  N   ILE A 144      60.476  -1.418  20.564  1.00 20.33           N
ATOM   1262  CA  ILE A 144      59.725  -2.314  21.407  1.00 15.35           C
ATOM   1263  C   ILE A 144      59.706  -3.732  20.859  1.00 19.84           C
ATOM   1264  O   ILE A 144      59.836  -4.700  21.608  1.00 17.93           O
ATOM   1265  CB  ILE A 144      58.317  -1.819  21.559  1.00 10.60           C
ATOM   1266  CG1 ILE A 144      58.311  -0.610  22.516  1.00  9.80           C
ATOM   1267  CG2 ILE A 144      57.410  -2.928  22.122  1.00  9.60           C
ATOM   1268  CD1 ILE A 144      57.022   0.076  22.517  1.00 18.32           C
ATOM   1269  N   LYS A 145      59.520  -3.841  19.556  1.00  7.20           N
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ATOM	1270	CA	LYS A 145	59.459	-5.139	18.926	1.00	7.64		C
ATOM	1271	C	LYS A 145	60.840	-5.788	18.931	1.00	15.32		C
ATOM	1272	O	LYS A 145	60.923	-6.989	18.981	1.00	14.76		O
ATOM	1273	CB	LYS A 145	58.891	-5.001	17.516	1.00	11.25		C
ATOM	1274	CG	LYS A 145	57.414	-4.581	17.489	1.00	12.13		C
ATOM	1275	CD	LYS A 145	56.642	-5.434	18.495	1.00	25.23		C
ATOM	1276	CE	LYS A 145	55.189	-4.995	18.692	1.00	13.64		C
ATOM	1277	NZ	LYS A 145	54.441	-6.111	19.392	1.00	11.94		N
ATOM	1278	N	LEU A 146	61.934	-5.011	18.986	1.00	26.98		N
ATOM	1279	CA	LEU A 146	63.261	-5.642	19.167	1.00	19.72		C
ATOM	1280	C	LEU A 146	63.262	-6.316	20.542	1.00	18.20		C
ATOM	1281	O	LEU A 146	63.590	-7.511	20.703	1.00	19.86		O
ATOM	1282	CB	LEU A 146	64.398	-4.618	19.150	1.00	13.56		C
ATOM	1283	CG	LEU A 146	64.895	-4.258	17.759	1.00	21.84		C
ATOM	1284	CD1	LEU A 146	65.672	-2.945	17.817	1.00	17.94		C
ATOM	1285	CD2	LEU A 146	65.745	-5.397	17.102	1.00	16.10		C
ATOM	1286	N	CYS A 147	62.931	-5.523	21.548	1.00	7.91		N
ATOM	1287	CA	CYS A 147	62.875	-6.064	22.893	1.00	9.14		C
ATOM	1288	C	CYS A 147	62.072	-7.378	22.945	1.00	22.72		C
ATOM	1289	O	CYS A 147	62.568	-8.401	23.383	1.00	16.90		O
ATOM	1290	CB	CYS A 147	62.232	-5.058	23.809	1.00	12.63		C
ATOM	1291	SG	CYS A 147	63.411	-3.823	24.316	1.00	15.02		S
ATOM	1292	N	GLU A 148	60.823	-7.352	22.508	1.00	20.03		N
ATOM	1293	CA	GLU A 148	60.016	-8.555	22.567	1.00	16.09		C
ATOM	1294	C	GLU A 148	60.685	-9.715	21.802	1.00	22.61		C
ATOM	1295	O	GLU A 148	60.651	-10.888	22.226	1.00	12.05		O
ATOM	1296	CB	GLU A 148	58.597	-8.268	22.046	1.00	14.66		C
ATOM	1297	CG	GLU A 148	57.864	-7.189	22.840	1.00	11.45		C
ATOM	1298	CD	GLU A 148	56.471	-6.821	22.277	1.00	11.75		C
ATOM	1299	OE1	GLU A 148	56.117	-7.055	21.080	1.00	11.65		O
ATOM	1300	OE2	GLU A 148	55.728	-6.231	23.081	1.00	22.56		O
ATOM	1301	N	SER A 149	61.368	-9.377	20.715	1.00	15.57		N
ATOM	1302	CA	SER A 149	61.938	-10.428	19.887	1.00	10.21		C
ATOM	1303	C	SER A 149	63.040	-11.245	20.502	1.00	15.83		C
ATOM	1304	O	SER A 149	63.102	-12.458	20.291	1.00	12.72		O
ATOM	1305	CB	SER A 149	62.270	-9.936	18.488	1.00	9.44		C
ATOM	1306	OG	SER A 149	61.053	-9.650	17.782	1.00	15.91		O
ATOM	1307	N	TYR A 150	63.910	-10.546	21.224	1.00	18.44		N
ATOM	1308	CA	TYR A 150	65.065	-11.100	21.948	1.00	20.50		C
ATOM	1309	C	TYR A 150	64.514	-11.848	23.158	1.00	21.87		C
ATOM	1310	O	TYR A 150	64.939	-12.949	23.486	1.00	31.39		O
ATOM	1311	CB	TYR A 150	66.005	-9.950	22.425	1.00	13.71		C
ATOM	1312	CG	TYR A 150	66.994	-9.509	21.365	1.00	14.13		C
ATOM	1313	CD1	TYR A 150	66.611	-8.673	20.317	1.00	14.64		C
ATOM	1314	CD2	TYR A 150	68.288	-10.000	21.360	1.00	18.32		C

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ATOM  1315  CE1 TYR A 150      67.487  -8.390  19.278  1.00 11.91           C
ATOM  1316  CE2 TYR A 150      69.198  -9.682  20.345  1.00 11.10           C
ATOM  1317  CZ  TYR A 150      68.804  -8.900  19.326  1.00 20.95           C
ATOM  1318  OH  TYR A 150      69.739  -8.685  18.333  1.00 27.73           O
ATOM  1319  N   ASN A 151      63.536 -11.249  23.801  1.00 14.83           N
ATOM  1320  CA  ASN A 151      62.903 -11.889  24.937  1.00 23.62           C
ATOM  1321  C   ASN A 151      62.417 -13.244  24.410  1.00 28.53           C
ATOM  1322  O   ASN A 151      62.630 -14.248  25.072  1.00 25.89           O
ATOM  1323  CB  ASN A 151      61.655 -11.113  25.439  1.00 20.95           C
ATOM  1324  CG  ASN A 151      61.988  -9.867  26.284  1.00 15.07           C
ATOM  1325  OD1 ASN A 151      61.126  -9.020  26.466  1.00 26.72           O
ATOM  1326  ND2 ASN A 151      63.231  -9.709  26.700  1.00  6.31           N
ATOM  1327  N   ARG A 152      61.731 -13.249  23.259  1.00 19.91           N
ATOM  1328  CA  ARG A 152      61.129 -14.465  22.687  1.00 17.62           C
ATOM  1329  C   ARG A 152      62.090 -15.523  22.188  1.00 21.34           C
ATOM  1330  O   ARG A 152      61.959 -16.687  22.542  1.00 15.44           O
ATOM  1331  CB  ARG A 152      60.086 -14.148  21.610  1.00 15.30           C
ATOM  1332  CG  ARG A 152      58.672 -13.754  22.157  1.00 17.22           C
ATOM  1333  CD  ARG A 152      57.652 -13.297  21.049  1.00  9.11           C
ATOM  1334  NE  ARG A 152      57.161 -14.419  20.241  1.00 21.05           N
ATOM  1335  CZ  ARG A 152      57.159 -14.447  18.912  1.00 28.61           C
ATOM  1336  NH1 ARG A 152      57.590 -13.387  18.221  1.00 21.98           N
ATOM  1337  NH2 ARG A 152      56.717 -15.528  18.262  1.00 26.11           N
ATOM  1338  N   GLN A 153      63.098 -15.104  21.434  1.00 16.54           N
ATOM  1339  CA  GLN A 153      64.044 -16.036  20.842  1.00  9.74           C
ATOM  1340  C   GLN A 153      65.082 -16.443  21.807  1.00 16.70           C
ATOM  1341  O   GLN A 153      65.529 -17.545  21.763  1.00 24.35           O
ATOM  1342  CB  GLN A 153      64.789 -15.372  19.714  1.00  8.99           C
ATOM  1343  CG  GLN A 153      65.935 -16.225  19.116  1.00  4.63           C
ATOM  1344  CD  GLN A 153      66.315 -15.637  17.762  1.00 14.17           C
ATOM  1345  OE1 GLN A 153      65.611 -14.763  17.254  1.00 12.53           O
ATOM  1346  NE2 GLN A 153      67.466 -16.024  17.228  1.00 13.38           N
ATOM  1347  N   TYR A 154      65.566 -15.518  22.608  1.00 14.35           N
ATOM  1348  CA  TYR A 154      66.677 -15.839  23.483  1.00 12.16           C
ATOM  1349  C   TYR A 154      66.323 -15.930  24.954  1.00 19.06           C
ATOM  1350  O   TYR A 154      67.185 -16.207  25.777  1.00 25.59           O
ATOM  1351  CB  TYR A 154      67.829 -14.816  23.326  1.00 16.89           C
ATOM  1352  CG  TYR A 154      68.418 -14.733  21.943  1.00 17.53           C
ATOM  1353  CD1 TYR A 154      69.259 -15.726  21.467  1.00 18.91           C
ATOM  1354  CD2 TYR A 154      68.080 -13.712  21.091  1.00 13.97           C
ATOM  1355  CE1 TYR A 154      69.782 -15.686  20.190  1.00 10.98           C
ATOM  1356  CE2 TYR A 154      68.621 -13.639  19.806  1.00 23.81           C
ATOM  1357  CZ  TYR A 154      69.488 -14.634  19.380  1.00 23.08           C
ATOM  1358  OH  TYR A 154      70.002 -14.619  18.118  1.00 23.87           O
ATOM  1359  N   GLY A 155      65.080 -15.686  25.313  1.00 12.08           N
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ATOM   1360  CA  GLY A 155      64.747 -15.702  26.731  1.00 15.80           C
ATOM   1361  C   GLY A 155      65.323 -14.498  27.580  1.00 33.97           C
ATOM   1362  O   GLY A 155      65.491 -14.640  28.789  1.00 25.76           O
ATOM   1363  N   ARG A 156      65.564 -13.318  26.981  1.00 25.91           N
ATOM   1364  CA  ARG A 156      66.066 -12.146  27.734  1.00 14.13           C
ATOM   1365  C   ARG A 156      64.971 -11.486  28.581  1.00 16.23           C
ATOM   1366  O   ARG A 156      63.802 -11.919  28.583  1.00 22.61           O
ATOM   1367  CB  ARG A 156      66.601 -11.124  26.750  1.00 13.16           C
ATOM   1368  CG  ARG A 156      67.875 -11.570  26.099  1.00 15.18           C
ATOM   1369  CD  ARG A 156      68.930 -11.418  27.121  1.00 26.42           C
ATOM   1370  NE  ARG A 156      70.200 -11.912  26.633  1.00 21.25           N
ATOM   1371  CZ  ARG A 156      71.092 -12.555  27.386  1.00 42.25           C
ATOM   1372  NH1 ARG A 156      70.870 -12.795  28.679  1.00 20.02           N
ATOM   1373  NH2 ARG A 156      72.221 -12.966  26.843  1.00 20.88           N
ATOM   1374  N   ASP A 157      65.343 -10.446  29.321  1.00 16.00           N
ATOM   1375  CA  ASP A 157      64.370  -9.749  30.166  1.00 16.20           C
ATOM   1376  C   ASP A 157      64.444  -8.245  29.841  1.00 19.20           C
ATOM   1377  O   ASP A 157      64.865  -7.429  30.650  1.00 10.71           O
ATOM   1378  CB  ASP A 157      64.609 -10.061  31.652  1.00 16.50           C
ATOM   1379  CG  ASP A 157      63.489  -9.560  32.566  1.00 26.45           C
ATOM   1380  OD1 ASP A 157      62.433  -9.060  32.108  1.00 26.82           O
ATOM   1381  OD2 ASP A 157      63.673  -9.653  33.784  1.00 21.88           O
ATOM   1382  N   TYR A 158      64.038  -7.921  28.620  1.00 19.41           N
ATOM   1383  CA  TYR A 158      64.099  -6.564  28.083  1.00 18.96           C
ATOM   1384  C   TYR A 158      62.688  -5.977  28.127  1.00 22.62           C
ATOM   1385  O   TYR A 158      61.854  -6.296  27.282  1.00 10.12           O
ATOM   1386  CB  TYR A 158      64.562  -6.661  26.631  1.00 16.34           C
ATOM   1387  CG  TYR A 158      65.982  -7.166  26.484  1.00 12.04           C
ATOM   1388  CD1 TYR A 158      66.789  -7.415  27.621  1.00 13.76           C
ATOM   1389  CD2 TYR A 158      66.544  -7.349  25.218  1.00 16.35           C
ATOM   1390  CE1 TYR A 158      68.135  -7.786  27.482  1.00  8.18           C
ATOM   1391  CE2 TYR A 158      67.886  -7.732  25.060  1.00 13.73           C
ATOM   1392  CZ  TYR A 158      68.676  -7.942  26.186  1.00 24.45           C
ATOM   1393  OH  TYR A 158      69.993  -8.338  25.997  1.00 14.36           O
ATOM   1394  N   ARG A 159      62.423  -5.200  29.175  1.00 23.53           N
ATOM   1395  CA  ARG A 159      61.105  -4.603  29.483  1.00 21.15           C
ATOM   1396  C   ARG A 159      60.930  -3.172  28.878  1.00 23.55           C
ATOM   1397  O   ARG A 159      61.911  -2.566  28.424  1.00 18.12           O
ATOM   1398  CB  ARG A 159      60.891  -4.608  31.034  1.00 21.68           C
ATOM   1399  CG  ARG A 159      60.986  -6.029  31.722  1.00 16.41           C
ATOM   1400  CD  ARG A 159      61.135  -6.052  33.233  1.00 18.10           C
ATOM   1401  NE  ARG A 159      61.305  -7.402  33.772  1.00 19.25           N
ATOM   1402  CZ  ARG A 159      61.164  -7.720  35.058  1.00 36.67           C
ATOM   1403  NH1 ARG A 159      60.886  -6.776  35.962  1.00 15.32           N
ATOM   1404  NH2 ARG A 159      61.309  -8.986  35.448  1.00 11.79           N
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ATOM	1405	N	SER A 160	59.689	-2.661	28.859	1.00	24.44		N
ATOM	1406	CA	SER A 160	59.312	-1.393	28.200	1.00	21.59		C
ATOM	1407	C	SER A 160	58.242	-0.577	28.950	1.00	25.07		C
ATOM	1408	O	SER A 160	57.257	-1.127	29.454	1.00	17.02		O
ATOM	1409	CB	SER A 160	58.719	-1.747	26.797	1.00	13.05		C
ATOM	1410	OG	SER A 160	59.782	-1.897	25.885	1.00	37.57		O
ATOM	1411	N	VAL A 161	58.378	0.742	28.927	1.00	21.01		N
ATOM	1412	CA	VAL A 161	57.369	1.644	29.509	1.00	9.70		C
ATOM	1413	C	VAL A 161	57.068	2.747	28.504	1.00	16.77		C
ATOM	1414	O	VAL A 161	57.955	3.149	27.729	1.00	16.33		O
ATOM	1415	CB	VAL A 161	57.806	2.248	30.862	1.00	17.94		C
ATOM	1416	CG1	VAL A 161	57.873	1.185	31.984	1.00	16.16		C
ATOM	1417	CG2	VAL A 161	59.137	2.992	30.750	1.00	21.10		C
ATOM	1418	N	MET A 162	55.794	3.147	28.443	1.00	22.46		N
ATOM	1419	CA	MET A 162	55.296	4.185	27.513	1.00	19.23		C
ATOM	1420	C	MET A 162	54.880	5.312	28.397	1.00	25.19		C
ATOM	1421	O	MET A 162	53.788	5.269	28.961	1.00	18.35		O
ATOM	1422	CB	MET A 162	53.979	3.796	26.850	1.00	15.55		C
ATOM	1423	CG	MET A 162	54.013	2.630	25.949	1.00	37.79		C
ATOM	1424	SD	MET A 162	54.354	3.100	24.235	1.00	52.07		S
ATOM	1425	CE	MET A 162	56.193	3.134	24.410	1.00	36.30		C
ATOM	1426	N	PRO A 163	55.730	6.313	28.521	1.00	18.43		N
ATOM	1427	CA	PRO A 163	55.390	7.472	29.337	1.00	17.76		C
ATOM	1428	C	PRO A 163	54.300	8.384	28.667	1.00	21.23		C
ATOM	1429	O	PRO A 163	54.208	8.448	27.433	1.00	15.20		O
ATOM	1430	CB	PRO A 163	56.727	8.196	29.423	1.00	11.43		C
ATOM	1431	CG	PRO A 163	57.352	7.874	28.031	1.00	13.99		C
ATOM	1432	CD	PRO A 163	57.086	6.401	27.949	1.00	12.24		C
ATOM	1433	N	THR A 164	53.478	9.060	29.478	1.00	13.95		N
ATOM	1434	CA	THR A 164	52.581	10.121	28.963	1.00	25.82		C
ATOM	1435	C	THR A 164	53.406	11.441	28.781	1.00	19.67		C
ATOM	1436	O	THR A 164	54.633	11.393	28.868	1.00	13.97		O
ATOM	1437	CB	THR A 164	51.373	10.391	29.903	1.00	25.51		C
ATOM	1438	OG1	THR A 164	50.470	11.321	29.267	1.00	14.77		O
ATOM	1439	CG2	THR A 164	51.818	10.886	31.298	1.00	9.06		C
ATOM	1440	N	ASN A 165	52.751	12.589	28.556	1.00	14.99		N
ATOM	1441	CA	ASN A 165	53.448	13.901	28.481	1.00	7.83		C
ATOM	1442	C	ASN A 165	54.167	14.064	29.824	1.00	11.21		C
ATOM	1443	O	ASN A 165	53.554	13.929	30.894	1.00	17.66		O
ATOM	1444	CB	ASN A 165	52.434	15.061	28.416	1.00	14.48		C
ATOM	1445	CG	ASN A 165	51.492	14.941	27.262	1.00	23.70		C
ATOM	1446	OD1	ASN A 165	51.939	14.800	26.129	1.00	22.37		O
ATOM	1447	ND2	ASN A 165	50.173	14.925	27.539	1.00	27.22		N
ATOM	1448	N	LEU A 166	55.418	14.490	29.777	1.00	8.23		N
ATOM	1449	CA	LEU A 166	56.187	14.604	30.994	1.00	14.40		C

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ATOM   1450  C   LEU A 166      56.629  16.017  31.120  1.00 25.05           C
ATOM   1451  O   LEU A 166      56.624  16.718  30.125  1.00 25.09           O
ATOM   1452  CB  LEU A 166      57.460  13.743  30.870  1.00 17.48           C
ATOM   1453  CG  LEU A 166      57.423  12.218  30.652  1.00 16.63           C
ATOM   1454  CD1 LEU A 166      58.837  11.639  31.000  1.00 22.52           C
ATOM   1455  CD2 LEU A 166      56.336  11.539  31.514  1.00  7.46           C
ATOM   1456  N   TYR A 167      57.146  16.391  32.300  1.00 19.78           N
ATOM   1457  CA  TYR A 167      57.678  17.760  32.511  1.00 18.58           C
ATOM   1458  C   TYR A 167      58.534  17.763  33.767  1.00 15.53           C
ATOM   1459  O   TYR A 167      58.474  16.852  34.575  1.00 16.71           O
ATOM   1460  CB  TYR A 167      56.509  18.778  32.665  1.00 18.33           C
ATOM   1461  CG  TYR A 167      55.671  18.561  33.931  1.00 14.23           C
ATOM   1462  CD1 TYR A 167      54.624  17.618  33.977  1.00 13.35           C
ATOM   1463  CD2 TYR A 167      55.984  19.258  35.106  1.00 16.52           C
ATOM   1464  CE1 TYR A 167      53.889  17.446  35.146  1.00 21.17           C
ATOM   1465  CE2 TYR A 167      55.302  19.084  36.264  1.00  8.26           C
ATOM   1466  CZ  TYR A 167      54.228  18.203  36.296  1.00 23.56           C
ATOM   1467  OH  TYR A 167      53.526  18.078  37.504  1.00 22.81           O
ATOM   1468  N   GLY A 168      59.334  18.797  33.952  1.00 16.59           N
ATOM   1469  CA  GLY A 168      60.158  18.817  35.152  1.00 18.21           C
ATOM   1470  C   GLY A 168      61.534  19.428  34.880  1.00 13.69           C
ATOM   1471  O   GLY A 168      61.746  20.028  33.837  1.00 16.52           O
ATOM   1472  N   PRO A 169      62.473  19.263  35.817  1.00 20.33           N
ATOM   1473  CA  PRO A 169      63.801  19.822  35.656  1.00 16.07           C
ATOM   1474  C   PRO A 169      64.430  19.353  34.387  1.00 27.18           C
ATOM   1475  O   PRO A 169      64.305  18.186  33.981  1.00 21.23           O
ATOM   1476  CB  PRO A 169      64.595  19.206  36.805  1.00 17.28           C
ATOM   1477  CG  PRO A 169      63.649  18.919  37.830  1.00 19.89           C
ATOM   1478  CD  PRO A 169      62.263  18.772  37.189  1.00 22.47           C
ATOM   1479  N   HIS A 170      65.226  20.235  33.829  1.00 19.48           N
ATOM   1480  CA  HIS A 170      65.952  19.877  32.638  1.00 25.56           C
ATOM   1481  C   HIS A 170      65.096  19.707  31.428  1.00 29.15           C
ATOM   1482  O   HIS A 170      65.553  19.091  30.479  1.00 29.71           O
ATOM   1483  CB  HIS A 170      66.783  18.600  32.845  1.00 28.94           C
ATOM   1484  CG  HIS A 170      67.703  18.671  34.034  1.00 33.88           C
ATOM   1485  ND1 HIS A 170      68.975  19.203  33.969  1.00 25.46           N
ATOM   1486  CD2 HIS A 170      67.518  18.298  35.326  1.00 34.77           C
ATOM   1487  CE1 HIS A 170      69.531  19.151  35.166  1.00 25.63           C
ATOM   1488  NE2 HIS A 170      68.673  18.603  36.008  1.00 31.72           N
ATOM   1489  N   ASP A 171      63.881  20.245  31.440  1.00 21.52           N
ATOM   1490  CA  ASP A 171      63.041  20.267  30.218  1.00 28.63           C
ATOM   1491  C   ASP A 171      63.630  21.459  29.359  1.00 41.94           C
ATOM   1492  O   ASP A 171      64.534  22.171  29.835  1.00 29.69           O
ATOM   1493  CB  ASP A 171      61.552  20.558  30.602  1.00 26.40           C
ATOM   1494  CG  ASP A 171      60.552  20.097  29.540  1.00 22.32           C
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ATOM	1495	OD1	ASP	A	171	60.890	20.067	28.325	1.00 32.03	O
ATOM	1496	OD2	ASP	A	171	59.427	19.719	29.916	1.00 42.13	O
ATOM	1497	N	ASN	A	172	63.141	21.712	28.137	1.00 42.08	N
ATOM	1498	CA	ASN	A	172	63.616	22.893	27.388	1.00 35.95	C
ATOM	1499	C	ASN	A	172	62.665	24.056	27.674	1.00 33.71	C
ATOM	1500	O	ASN	A	172	61.586	24.102	27.104	1.00 32.69	O
ATOM	1501	CB	ASN	A	172	63.632	22.667	25.869	1.00 41.60	C
ATOM	1502	CG	ASN	A	172	63.807	23.987	25.086	1.00 39.09	C
ATOM	1503	OD1	ASN	A	172	62.973	24.347	24.259	1.00 83.94	O
ATOM	1504	ND2	ASN	A	172	64.855	24.740	25.418	1.00 65.07	N
ATOM	1505	N	PHE	A	173	63.021	24.953	28.583	1.00 31.93	N
ATOM	1506	CA	PHE	A	173	62.082	26.030	28.944	1.00 48.24	C
ATOM	1507	C	PHE	A	173	61.989	27.260	28.045	1.00 69.01	C
ATOM	1508	O	PHE	A	173	62.278	28.395	28.465	1.00 58.79	O
ATOM	1509	CB	PHE	A	173	62.225	26.459	30.390	1.00 43.43	C
ATOM	1510	CG	PHE	A	173	61.867	25.399	31.356	1.00 34.19	C
ATOM	1511	CD1	PHE	A	173	62.810	24.488	31.751	1.00 24.68	C
ATOM	1512	CD2	PHE	A	173	60.621	25.354	31.925	1.00 24.84	C
ATOM	1513	CE1	PHE	A	173	62.524	23.548	32.682	1.00 23.64	C
ATOM	1514	CE2	PHE	A	173	60.305	24.366	32.804	1.00 31.32	C
ATOM	1515	CZ	PHE	A	173	61.263	23.457	33.192	1.00 24.30	C
ATOM	1516	N	HIS	A	174	61.510	27.036	26.831	1.00 68.16	N
ATOM	1517	CA	HIS	A	174	61.401	28.109	25.871	1.00 64.53	C
ATOM	1518	C	HIS	A	174	59.973	28.221	25.400	1.00 71.58	C
ATOM	1519	O	HIS	A	174	59.309	27.186	25.249	1.00 73.20	O
ATOM	1520	CB	HIS	A	174	62.418	27.870	24.736	1.00 71.71	C
ATOM	1521	CG	HIS	A	174	63.835	27.868	25.229	1.00 92.29	C
ATOM	1522	ND1	HIS	A	174	64.921	27.539	24.440	1.00100.00	N
ATOM	1523	CD2	HIS	A	174	64.338	28.133	26.463	1.00100.00	C
ATOM	1524	CE1	HIS	A	174	66.032	27.628	25.160	1.00100.00	C
ATOM	1525	NE2	HIS	A	174	65.705	27.981	26.393	1.00100.00	N
ATOM	1526	N	PRO	A	175	59.469	29.461	25.262	1.00 65.71	N
ATOM	1527	CA	PRO	A	175	58.109	29.658	24.770	1.00 55.72	C
ATOM	1528	C	PRO	A	175	58.233	29.297	23.267	1.00 75.83	C
ATOM	1529	O	PRO	A	175	57.224	29.226	22.554	1.00 69.59	O
ATOM	1530	CB	PRO	A	175	57.866	31.142	25.026	1.00 49.14	C
ATOM	1531	CG	PRO	A	175	59.258	31.790	24.901	1.00 42.23	C
ATOM	1532	CD	PRO	A	175	60.286	30.695	25.109	1.00 49.59	C
ATOM	1533	N	SER	A	176	59.480	28.954	22.879	1.00 85.09	N
ATOM	1534	CA	SER	A	176	59.954	28.474	21.548	1.00 81.18	C
ATOM	1535	C	SER	A	176	59.660	26.965	21.343	1.00 73.90	C
ATOM	1536	O	SER	A	176	59.617	26.458	20.213	1.00 57.03	O
ATOM	1537	CB	SER	A	176	61.493	28.666	21.447	1.00 71.32	C
ATOM	1538	OG	SER	A	176	62.048	29.349	22.578	1.00 51.93	O
ATOM	1539	N	ASN	A	177	59.520	26.276	22.480	1.00 66.23	N

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ATOM   1540  CA  ASN A 177      59.274  24.847  22.619  1.00 56.41           C
ATOM   1541  C   ASN A 177      57.810  24.497  22.353  1.00 60.91           C
ATOM   1542  O   ASN A 177      56.914  25.215  22.811  1.00 55.58           O
ATOM   1543  CB  ASN A 177      59.619  24.469  24.065  1.00 50.45           C
ATOM   1544  CG  ASN A 177      59.562  22.970  24.319  1.00 66.57           C
ATOM   1545  OD1 ASN A 177      59.095  22.216  23.476  1.00100.00           O
ATOM   1546  ND2 ASN A 177      60.099  22.546  25.464  1.00 35.61           N
ATOM   1547  N   SER A 178      57.583  23.387  21.627  1.00 57.10           N
ATOM   1548  CA  SER A 178      56.234  22.853  21.279  1.00 50.50           C
ATOM   1549  C   SER A 178      55.557  22.159  22.491  1.00 76.24           C
ATOM   1550  O   SER A 178      54.575  21.400  22.304  1.00 99.63           O
ATOM   1551  CB  SER A 178      56.316  21.800  20.118  1.00 10.17           C
ATOM   1552  OG  SER A 178      57.397  22.112  19.217  1.00 71.69           O
ATOM   1553  N   HIS A 179      56.134  22.284  23.694  1.00 37.39           N
ATOM   1554  CA  HIS A 179      55.569  21.587  24.855  1.00 30.96           C
ATOM   1555  C   HIS A 179      54.961  22.616  25.767  1.00 21.93           C
ATOM   1556  O   HIS A 179      55.641  23.598  26.138  1.00 25.17           O
ATOM   1557  CB  HIS A 179      56.634  20.683  25.575  1.00 36.20           C
ATOM   1558  CG  HIS A 179      56.973  19.419  24.835  1.00 42.90           C
ATOM   1559  ND1 HIS A 179      56.973  19.335  23.457  1.00 49.52           N
ATOM   1560  CD2 HIS A 179      57.323  18.190  25.278  1.00 52.42           C
ATOM   1561  CE1 HIS A 179      57.283  18.109  23.084  1.00 44.78           C
ATOM   1562  NE2 HIS A 179      57.500  17.393  24.168  1.00 50.49           N
ATOM   1563  N   VAL A 180      53.661  22.454  26.038  1.00 19.14           N
ATOM   1564  CA  VAL A 180      52.886  23.449  26.789  1.00 29.03           C
ATOM   1565  C   VAL A 180      53.373  23.890  28.142  1.00 31.29           C
ATOM   1566  O   VAL A 180      53.348  25.075  28.447  1.00 19.55           O
ATOM   1567  CB  VAL A 180      51.403  23.115  26.914  1.00 35.47           C
ATOM   1568  CG1 VAL A 180      50.630  24.399  27.217  1.00 35.84           C
ATOM   1569  CG2 VAL A 180      50.923  22.550  25.663  1.00 36.11           C
ATOM   1570  N   ILE A 181      53.684  22.935  29.005  1.00 26.57           N
ATOM   1571  CA  ILE A 181      54.138  23.285  30.360  1.00 24.49           C
ATOM   1572  C   ILE A 181      55.371  24.213  30.361  1.00 16.51           C
ATOM   1573  O   ILE A 181      55.326  25.315  30.909  1.00 24.42           O
ATOM   1574  CB  ILE A 181      54.285  22.018  31.264  1.00 20.20           C
ATOM   1575  CG1 ILE A 181      52.878  21.428  31.528  1.00 18.22           C
ATOM   1576  CG2 ILE A 181      55.014  22.315  32.581  1.00 13.37           C
ATOM   1577  CD1 ILE A 181      52.867  20.086  32.286  1.00  8.03           C
ATOM   1578  N   PRO A 182      56.452  23.779  29.718  1.00 22.21           N
ATOM   1579  CA  PRO A 182      57.664  24.605  29.640  1.00 22.07           C
ATOM   1580  C   PRO A 182      57.379  25.852  28.828  1.00 24.18           C
ATOM   1581  O   PRO A 182      57.811  26.949  29.210  1.00 18.35           O
ATOM   1582  CB  PRO A 182      58.682  23.725  28.890  1.00 24.97           C
ATOM   1583  CG  PRO A 182      57.925  22.473  28.471  1.00 25.77           C
ATOM   1584  CD  PRO A 182      56.727  22.359  29.401  1.00 18.23           C
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ATOM   1585  N   ALA A 183      56.628  25.707  27.729  1.00 21.45           N
ATOM   1586  CA  ALA A 183      56.261  26.896  26.943  1.00 21.66           C
ATOM   1587  C   ALA A 183      55.464  27.900  27.811  1.00 26.10           C
ATOM   1588  O   ALA A 183      55.773  29.091  27.856  1.00 19.50           O
ATOM   1589  CB  ALA A 183      55.473  26.513  25.703  1.00 13.26           C
ATOM   1590  N   LEU A 184      54.472  27.389  28.543  1.00 23.34           N
ATOM   1591  CA  LEU A 184      53.642  28.215  29.401  1.00 19.05           C
ATOM   1592  C   LEU A 184      54.312  28.693  30.655  1.00 21.91           C
ATOM   1593  O   LEU A 184      54.017  29.771  31.158  1.00 19.71           O
ATOM   1594  CB  LEU A 184      52.309  27.553  29.715  1.00 14.41           C
ATOM   1595  CG  LEU A 184      51.342  27.595  28.525  1.00 23.42           C
ATOM   1596  CD1 LEU A 184      49.918  27.244  28.928  1.00 31.06           C
ATOM   1597  CD2 LEU A 184      51.380  28.896  27.690  1.00 21.73           C
ATOM   1598  N   LEU A 185      55.178  27.879  31.213  1.00 18.39           N
ATOM   1599  CA  LEU A 185      55.833  28.332  32.417  1.00 16.39           C
ATOM   1600  C   LEU A 185      56.669  29.528  31.985  1.00 23.67           C
ATOM   1601  O   LEU A 185      56.681  30.590  32.644  1.00 29.38           O
ATOM   1602  CB  LEU A 185      56.723  27.233  33.015  1.00 15.05           C
ATOM   1603  CG  LEU A 185      56.021  26.348  34.041  1.00 15.56           C
ATOM   1604  CD1 LEU A 185      56.819  25.022  34.301  1.00 21.06           C
ATOM   1605  CD2 LEU A 185      55.722  27.113  35.321  1.00 11.02           C
ATOM   1606  N   ARG A 186      57.337  29.397  30.852  1.00 17.09           N
ATOM   1607  CA  ARG A 186      58.137  30.523  30.429  1.00 18.82           C
ATOM   1608  C   ARG A 186      57.308  31.752  30.069  1.00 29.00           C
ATOM   1609  O   ARG A 186      57.629  32.880  30.476  1.00 23.91           O
ATOM   1610  CB  ARG A 186      59.026  30.146  29.281  1.00 22.06           C
ATOM   1611  CG  ARG A 186      59.653  31.365  28.652  1.00 38.46           C
ATOM   1612  CD  ARG A 186      60.825  31.804  29.462  1.00 83.66           C
ATOM   1613  NE  ARG A 186      62.012  31.861  28.631  1.00 70.77           N
ATOM   1614  CZ  ARG A 186      63.058  32.622  28.904  1.00 91.68           C
ATOM   1615  NH1 ARG A 186      63.053  33.386  29.995  1.00 56.56           N
ATOM   1616  NH2 ARG A 186      64.098  32.639  28.082  1.00100.00           N
ATOM   1617  N   ARG A 187      56.234  31.544  29.310  1.00 20.96           N
ATOM   1618  CA  ARG A 187      55.361  32.662  28.941  1.00 19.32           C
ATOM   1619  C   ARG A 187      54.765  33.453  30.142  1.00 28.41           C
ATOM   1620  O   ARG A 187      54.823  34.700  30.193  1.00 17.23           O
ATOM   1621  CB  ARG A 187      54.270  32.223  27.957  1.00 17.05           C
ATOM   1622  CG  ARG A 187      54.813  31.546  26.720  1.00 61.42           C
ATOM   1623  CD  ARG A 187      53.696  31.244  25.757  1.00 44.57           C
ATOM   1624  NE  ARG A 187      53.033  32.472  25.354  1.00 29.47           N
ATOM   1625  CZ  ARG A 187      51.831  32.534  24.790  1.00 17.82           C
ATOM   1626  NH1 ARG A 187      51.136  31.427  24.544  1.00 24.95           N
ATOM   1627  NH2 ARG A 187      51.341  33.716  24.447  1.00 37.77           N
ATOM   1628  N   PHE A 188      54.192  32.734  31.101  1.00 23.48           N
ATOM   1629  CA  PHE A 188      53.604  33.399  32.259  1.00 21.24           C
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ATOM	1630	C	PHE	A	188	54.638	34.080	33.095	1.00	21.39	C
ATOM	1631	O	PHE	A	188	54.394	35.126	33.626	1.00	23.90	O
ATOM	1632	CB	PHE	A	188	52.723	32.466	33.077	1.00	19.95	C
ATOM	1633	CG	PHE	A	188	51.389	32.215	32.435	1.00	22.28	C
ATOM	1634	CD1	PHE	A	188	50.440	33.229	32.375	1.00	19.42	C
ATOM	1635	CD2	PHE	A	188	51.144	31.038	31.734	1.00	23.82	C
ATOM	1636	CE1	PHE	A	188	49.191	33.026	31.742	1.00	24.77	C
ATOM	1637	CE2	PHE	A	188	49.936	30.826	31.057	1.00	20.17	C
ATOM	1638	CZ	PHE	A	188	48.945	31.815	31.068	1.00	23.14	C
ATOM	1639	N	HIS	A	189	55.831	33.513	33.118	1.00	24.15	N
ATOM	1640	CA	HIS	A	189	56.933	34.122	33.837	1.00	28.79	C
ATOM	1641	C	HIS	A	189	57.303	35.506	33.315	1.00	28.58	C
ATOM	1642	O	HIS	A	189	57.480	36.463	34.083	1.00	20.07	O
ATOM	1643	CB	HIS	A	189	58.148	33.268	33.641	1.00	31.38	C
ATOM	1644	CG	HIS	A	189	59.364	33.844	34.290	1.00	29.98	C
ATOM	1645	ND1	HIS	A	189	59.548	33.833	35.658	1.00	31.00	N
ATOM	1646	CD2	HIS	A	189	60.449	34.464	33.766	1.00	21.79	C
ATOM	1647	CE1	HIS	A	189	60.722	34.371	35.945	1.00	24.04	C
ATOM	1648	NE2	HIS	A	189	61.257	34.815	34.821	1.00	19.53	N
ATOM	1649	N	GLU	A	190	57.539	35.561	32.006	1.00	28.43	N
ATOM	1650	CA	GLU	A	190	57.876	36.816	31.324	1.00	27.72	C
ATOM	1651	C	GLU	A	190	56.725	37.829	31.437	1.00	32.56	C
ATOM	1652	O	GLU	A	190	56.949	38.995	31.717	1.00	27.06	O
ATOM	1653	CB	GLU	A	190	58.122	36.529	29.849	1.00	28.55	C
ATOM	1654	CG	GLU	A	190	59.150	35.461	29.614	1.00	35.29	C
ATOM	1655	CD	GLU	A	190	60.553	35.941	29.892	1.00	99.81	C
ATOM	1656	OE1	GLU	A	190	60.913	36.037	31.085	1.00	86.56	O
ATOM	1657	OE2	GLU	A	190	61.293	36.167	28.910	1.00	100.00	O
ATOM	1658	N	ALA	A	191	55.493	37.391	31.196	1.00	32.67	N
ATOM	1659	CA	ALA	A	191	54.349	38.286	31.311	1.00	25.30	C
ATOM	1660	C	ALA	A	191	54.287	38.795	32.742	1.00	36.20	C
ATOM	1661	O	ALA	A	191	53.920	39.924	33.014	1.00	27.52	O
ATOM	1662	CB	ALA	A	191	53.055	37.563	31.000	1.00	16.48	C
ATOM	1663	N	THR	A	192	54.549	37.927	33.693	1.00	29.39	N
ATOM	1664	CA	THR	A	192	54.395	38.386	35.041	1.00	19.08	C
ATOM	1665	C	THR	A	192	55.420	39.494	35.298	1.00	44.78	C
ATOM	1666	O	THR	A	192	55.094	40.550	35.839	1.00	40.58	O
ATOM	1667	CB	THR	A	192	54.515	37.235	35.983	1.00	18.99	C
ATOM	1668	OG1	THR	A	192	53.410	36.348	35.755	1.00	34.36	O
ATOM	1669	CG2	THR	A	192	54.461	37.738	37.425	1.00	21.15	C
ATOM	1670	N	ALA	A	193	56.617	39.312	34.757	1.00	48.58	N
ATOM	1671	CA	ALA	A	193	57.705	40.286	34.905	1.00	50.59	C
ATOM	1672	C	ALA	A	193	57.496	41.613	34.145	1.00	54.42	C
ATOM	1673	O	ALA	A	193	57.952	42.698	34.553	1.00	48.28	O
ATOM	1674	CB	ALA	A	193	59.047	39.640	34.496	1.00	51.78	C

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ATOM   1675  N   GLN A 194      56.810  41.530  33.022  1.00 43.16           N
ATOM   1676  CA  GLN A 194      56.586  42.722  32.242  1.00 38.03           C
ATOM   1677  C   GLN A 194      55.264  43.389  32.576  1.00 40.85           C
ATOM   1678  O   GLN A 194      54.830  44.284  31.845  1.00 51.20           O
ATOM   1679  CB  GLN A 194      56.599  42.358  30.750  1.00 35.96           C
ATOM   1680  CG  GLN A 194      57.910  41.692  30.290  1.00100.00           C
ATOM   1681  CD  GLN A 194      57.715  40.661  29.158  1.00100.00           C
ATOM   1682  OE1 GLN A 194      56.619  40.546  28.579  1.00100.00           O
ATOM   1683  NE2 GLN A 194      58.782  39.904  28.848  1.00100.00           N
ATOM   1684  N   GLY A 195      54.583  42.949  33.630  1.00 32.29           N
ATOM   1685  CA  GLY A 195      53.236  43.464  33.864  1.00 36.26           C
ATOM   1686  C   GLY A 195      52.299  43.332  32.593  1.00 45.33           C
ATOM   1687  O   GLY A 195      51.515  44.242  32.346  1.00 45.16           O
ATOM   1688  N   GLY A 196      52.405  42.245  31.788  1.00 36.33           N
ATOM   1689  CA  GLY A 196      51.515  41.965  30.608  1.00 19.06           C
ATOM   1690  C   GLY A 196      50.037  41.958  31.117  1.00 22.49           C
ATOM   1691  O   GLY A 196      49.724  41.479  32.223  1.00 33.09           O
ATOM   1692  N   PRO A 197      49.144  42.657  30.431  1.00 29.22           N
ATOM   1693  CA  PRO A 197      47.790  42.732  30.953  1.00 25.29           C
ATOM   1694  C   PRO A 197      47.091  41.413  30.674  1.00 24.64           C
ATOM   1695  O   PRO A 197      46.192  40.991  31.411  1.00 24.75           O
ATOM   1696  CB  PRO A 197      47.162  43.911  30.176  1.00 26.31           C
ATOM   1697  CG  PRO A 197      48.188  44.407  29.252  1.00 26.56           C
ATOM   1698  CD  PRO A 197      49.307  43.454  29.203  1.00 30.25           C
ATOM   1699  N   ASP A 198      47.572  40.723  29.658  1.00 16.88           N
ATOM   1700  CA  ASP A 198      47.067  39.418  29.405  1.00 21.65           C
ATOM   1701  C   ASP A 198      48.046  38.522  28.677  1.00 31.28           C
ATOM   1702  O   ASP A 198      49.062  38.978  28.172  1.00 34.57           O
ATOM   1703  CB  ASP A 198      45.739  39.507  28.669  1.00 32.80           C
ATOM   1704  CG  ASP A 198      45.868  40.055  27.256  1.00 46.13           C
ATOM   1705  OD1 ASP A 198      46.982  40.230  26.725  1.00 57.45           O
ATOM   1706  OD2 ASP A 198      44.817  40.271  26.640  1.00 67.61           O
ATOM   1707  N   VAL A 199      47.713  37.234  28.614  1.00 38.67           N
ATOM   1708  CA  VAL A 199      48.499  36.226  27.901  1.00 27.79           C
ATOM   1709  C   VAL A 199      47.462  35.469  27.065  1.00 25.88           C
ATOM   1710  O   VAL A 199      46.460  35.023  27.598  1.00 24.22           O
ATOM   1711  CB  VAL A 199      49.163  35.229  28.905  1.00 24.37           C
ATOM   1712  CG1 VAL A 199      49.874  34.047  28.160  1.00 20.28           C
ATOM   1713  CG2 VAL A 199      50.121  35.942  29.835  1.00 22.25           C
ATOM   1714  N   VAL A 200      47.661  35.386  25.757  1.00 23.72           N
ATOM   1715  CA  VAL A 200      46.701  34.694  24.903  1.00 23.99           C
ATOM   1716  C   VAL A 200      47.167  33.286  24.499  1.00 22.85           C
ATOM   1717  O   VAL A 200      48.321  33.108  24.188  1.00 29.77           O
ATOM   1718  CB  VAL A 200      46.358  35.548  23.680  1.00 23.11           C
ATOM   1719  CG1 VAL A 200      45.561  34.737  22.598  1.00 16.25           C
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ATOM 1720 CG2 VAL A 200 45.652 36.823 24.130 1.00 27.86 C
ATOM 1721 N VAL A 201 46.296 32.278 24.632 1.00 27.39 N
ATOM 1722 CA VAL A 201 46.588 30.893 24.265 1.00 9.63 C
ATOM 1723 C VAL A 201 45.653 30.529 23.165 1.00 19.63 C
ATOM 1724 O VAL A 201 44.452 30.755 23.312 1.00 17.61 O
ATOM 1725 CB VAL A 201 46.306 29.952 25.426 1.00 19.95 C
ATOM 1726 CG1 VAL A 201 46.703 28.519 25.054 1.00 20.85 C
ATOM 1727 CG2 VAL A 201 47.086 30.439 26.661 1.00 16.73 C
ATOM 1728 N TRP A 202 46.210 30.080 22.030 1.00 14.36 N
ATOM 1729 CA TRP A 202 45.422 29.693 20.865 1.00 18.97 C
ATOM 1730 C TRP A 202 44.495 28.572 21.313 1.00 36.22 C
ATOM 1731 O TRP A 202 44.934 27.694 22.057 1.00 31.46 O
ATOM 1732 CB TRP A 202 46.292 29.055 19.823 1.00 19.14 C
ATOM 1733 CG TRP A 202 47.243 29.894 19.066 1.00 33.65 C
ATOM 1734 CD1 TRP A 202 48.391 29.463 18.429 1.00 35.28 C
ATOM 1735 CD2 TRP A 202 47.126 31.282 18.772 1.00 39.90 C
ATOM 1736 NE1 TRP A 202 48.941 30.481 17.693 1.00 37.86 N
ATOM 1737 CE2 TRP A 202 48.228 31.624 17.922 1.00 38.35 C
ATOM 1738 CE3 TRP A 202 46.206 32.281 19.138 1.00 39.39 C
ATOM 1739 CZ2 TRP A 202 48.380 32.884 17.367 1.00 36.15 C
ATOM 1740 CZ3 TRP A 202 46.356 33.542 18.578 1.00 39.60 C
ATOM 1741 CH2 TRP A 202 47.428 33.828 17.684 1.00 40.99 C
ATOM 1742 N GLY A 203 43.245 28.564 20.842 1.00 25.59 N
ATOM 1743 CA GLY A 203 42.332 27.483 21.169 1.00 13.09 C
ATOM 1744 C GLY A 203 41.260 27.813 22.193 1.00 21.12 C
ATOM 1745 O GLY A 203 41.340 28.815 22.886 1.00 22.86 O
ATOM 1746 N SER A 204 40.270 26.919 22.262 1.00 16.88 N
ATOM 1747 CA SER A 204 39.163 26.979 23.192 1.00 18.36 C
ATOM 1748 C SER A 204 39.561 26.664 24.659 1.00 22.07 C
ATOM 1749 O SER A 204 38.888 27.096 25.604 1.00 34.39 O
ATOM 1750 CB SER A 204 38.053 25.998 22.740 1.00 9.99 C
ATOM 1751 OG SER A 204 38.237 24.695 23.291 1.00 16.37 O
ATOM 1752 N GLY A 205 40.562 25.813 24.854 1.00 12.42 N
ATOM 1753 CA GLY A 205 40.963 25.411 26.208 1.00 11.64 C
ATOM 1754 C GLY A 205 40.208 24.178 26.711 1.00 19.49 C
ATOM 1755 O GLY A 205 40.422 23.723 27.838 1.00 13.59 O
ATOM 1756 N THR A 206 39.292 23.683 25.881 1.00 15.38 N
ATOM 1757 CA THR A 206 38.432 22.594 26.281 1.00 10.80 C
ATOM 1758 C THR A 206 39.056 21.221 26.154 1.00 26.39 C
ATOM 1759 O THR A 206 38.564 20.267 26.737 1.00 23.28 O
ATOM 1760 CB THR A 206 37.124 22.562 25.460 1.00 12.86 C
ATOM 1761 OG1 THR A 206 37.438 22.395 24.082 1.00 13.12 O
ATOM 1762 CG2 THR A 206 36.348 23.840 25.620 1.00 10.62 C
ATOM 1763 N PRO A 207 40.101 21.083 25.354 1.00 21.10 N
ATOM 1764 CA PRO A 207 40.658 19.743 25.175 1.00 18.15 C

ATOM	1765	C	PRO A 207	41.316	19.181	26.423	1.00	21.75	C
ATOM	1766	O	PRO A 207	41.951	19.925	27.215	1.00	20.65	O
ATOM	1767	CB	PRO A 207	41.638	19.909	24.013	1.00	17.51	C
ATOM	1768	CG	PRO A 207	41.146	21.213	23.307	1.00	21.45	C
ATOM	1769	CD	PRO A 207	40.698	22.062	24.431	1.00	23.44	C
ATOM	1770	N	MET A 208	41.112	17.876	26.624	1.00	15.60	N
ATOM	1771	CA	MET A 208	41.694	17.167	27.775	1.00	22.94	C
ATOM	1772	C	MET A 208	43.058	16.427	27.579	1.00	21.90	C
ATOM	1773	O	MET A 208	43.248	15.677	26.633	1.00	23.16	O
ATOM	1774	CB	MET A 208	40.645	16.273	28.386	1.00	32.86	C
ATOM	1775	CG	MET A 208	39.630	17.057	29.223	1.00	46.17	C
ATOM	1776	SD	MET A 208	38.301	15.990	29.826	1.00	57.85	S
ATOM	1777	CE	MET A 208	37.999	15.028	28.343	1.00	58.23	C
ATOM	1778	N	ARG A 209	44.022	16.681	28.456	1.00	17.75	N
ATOM	1779	CA	ARG A 209	45.318	16.042	28.324	1.00	19.88	C
ATOM	1780	C	ARG A 209	45.871	15.534	29.639	1.00	16.92	C
ATOM	1781	O	ARG A 209	45.433	15.946	30.697	1.00	16.58	O
ATOM	1782	CB	ARG A 209	46.340	16.963	27.658	1.00	21.07	C
ATOM	1783	CG	ARG A 209	45.980	17.478	26.275	1.00	22.57	C
ATOM	1784	CD	ARG A 209	45.833	16.357	25.282	1.00	28.26	C
ATOM	1785	NE	ARG A 209	45.586	16.819	23.906	1.00	23.15	N
ATOM	1786	CZ	ARG A 209	44.420	16.742	23.267	1.00	34.52	C
ATOM	1787	NH1	ARG A 209	43.336	16.267	23.890	1.00	18.03	N
ATOM	1788	NH2	ARG A 209	44.339	17.175	22.012	1.00	29.78	N
ATOM	1789	N	GLU A 210	46.878	14.675	29.547	1.00	20.87	N
ATOM	1790	CA	GLU A 210	47.530	14.079	30.720	1.00	17.37	C
ATOM	1791	C	GLU A 210	49.031	14.490	30.851	1.00	20.96	C
ATOM	1792	O	GLU A 210	49.748	14.622	29.841	1.00	22.44	O
ATOM	1793	CB	GLU A 210	47.400	12.562	30.571	1.00	16.26	C
ATOM	1794	CG	GLU A 210	47.807	11.785	31.809	1.00	19.91	C
ATOM	1795	CD	GLU A 210	48.057	10.304	31.531	1.00	27.81	C
ATOM	1796	OE1	GLU A 210	48.111	9.919	30.343	1.00	17.29	O
ATOM	1797	OE2	GLU A 210	48.268	9.540	32.494	1.00	21.63	O
ATOM	1798	N	PHE A 211	49.504	14.712	32.084	1.00	14.02	N
ATOM	1799	CA	PHE A 211	50.887	15.159	32.353	1.00	17.48	C
ATOM	1800	C	PHE A 211	51.458	14.414	33.531	1.00	33.62	C
ATOM	1801	O	PHE A 211	50.716	14.031	34.443	1.00	27.96	O
ATOM	1802	CB	PHE A 211	50.933	16.677	32.644	1.00	17.78	C
ATOM	1803	CG	PHE A 211	50.303	17.490	31.541	1.00	21.49	C
ATOM	1804	CD1	PHE A 211	51.009	17.676	30.320	1.00	17.36	C
ATOM	1805	CD2	PHE A 211	48.933	17.844	31.618	1.00	15.09	C
ATOM	1806	CE1	PHE A 211	50.399	18.334	29.237	1.00	16.37	C
ATOM	1807	CE2	PHE A 211	48.288	18.491	30.533	1.00	9.61	C
ATOM	1808	CZ	PHE A 211	49.053	18.756	29.344	1.00	12.71	C
ATOM	1809	N	LEU A 212	52.761	14.161	33.495	1.00	23.76	N

ATOM 1810 CA LEU A 212 53.405 13.448 34.603 1.00 21.24 C
ATOM 1811 C LEU A 212 54.772 14.053 34.898 1.00 14.00 C
ATOM 1812 O LEU A 212 55.519 14.398 33.985 1.00 13.99 O
ATOM 1813 CB LEU A 212 53.548 11.954 34.294 1.00 21.52 C
ATOM 1814 CG LEU A 212 54.033 11.039 35.406 1.00 21.09 C
ATOM 1815 CD1 LEU A 212 52.866 10.634 36.280 1.00 20.84 C
ATOM 1816 CD2 LEU A 212 54.768 9.829 34.832 1.00 13.18 C
ATOM 1817 N HIS A 213 55.023 14.302 36.175 1.00 9.60 N
ATOM 1818 CA HIS A 213 56.290 14.864 36.555 1.00 13.66 C
ATOM 1819 C HIS A 213 57.380 13.828 36.293 1.00 20.37 C
ATOM 1820 O HIS A 213 57.238 12.614 36.542 1.00 16.08 O
ATOM 1821 CB HIS A 213 56.280 15.250 38.002 1.00 18.72 C
ATOM 1822 CG HIS A 213 57.491 16.017 38.408 1.00 21.22 C
ATOM 1823 ND1 HIS A 213 58.703 15.406 38.656 1.00 24.29 N
ATOM 1824 CD2 HIS A 213 57.716 17.353 38.499 1.00 23.67 C
ATOM 1825 CE1 HIS A 213 59.615 16.331 38.917 1.00 19.13 C
ATOM 1826 NE2 HIS A 213 59.041 17.523 38.847 1.00 21.99 N
ATOM 1827 N VAL A 214 58.459 14.295 35.698 1.00 21.07 N
ATOM 1828 CA VAL A 214 59.532 13.383 35.361 1.00 19.23 C
ATOM 1829 C VAL A 214 60.067 12.523 36.551 1.00 27.20 C
ATOM 1830 O VAL A 214 60.604 11.444 36.359 1.00 22.23 O
ATOM 1831 CB VAL A 214 60.625 14.125 34.566 1.00 11.84 C
ATOM 1832 CG1 VAL A 214 61.390 15.199 35.485 1.00 8.52 C
ATOM 1833 CG2 VAL A 214 61.560 13.097 33.902 1.00 12.39 C
ATOM 1834 N ASP A 215 59.893 12.984 37.790 1.00 25.29 N
ATOM 1835 CA ASP A 215 60.406 12.228 38.936 1.00 18.19 C
ATOM 1836 C ASP A 215 59.530 11.023 39.230 1.00 13.85 C
ATOM 1837 O ASP A 215 59.988 9.981 39.666 1.00 17.44 O
ATOM 1838 CB ASP A 215 60.575 13.129 40.155 1.00 16.27 C
ATOM 1839 CG ASP A 215 61.859 13.979 40.068 1.00 30.73 C
ATOM 1840 OD1 ASP A 215 62.782 13.614 39.308 1.00 23.02 O
ATOM 1841 OD2 ASP A 215 61.957 15.029 40.730 1.00 26.00 O
ATOM 1842 N ASP A 216 58.276 11.136 38.863 1.00 20.08 N
ATOM 1843 CA ASP A 216 57.378 10.017 39.016 1.00 18.78 C
ATOM 1844 C ASP A 216 57.761 9.083 37.894 1.00 23.56 C
ATOM 1845 O ASP A 216 57.715 7.880 38.026 1.00 20.79 O
ATOM 1846 CB ASP A 216 55.912 10.457 38.821 1.00 17.18 C
ATOM 1847 CG ASP A 216 55.193 10.757 40.162 1.00 38.03 C
ATOM 1848 OD1 ASP A 216 55.503 10.119 41.223 1.00 26.02 O
ATOM 1849 OD2 ASP A 216 54.249 11.587 40.124 1.00 25.41 O
ATOM 1850 N MET A 217 58.092 9.653 36.755 1.00 18.11 N
ATOM 1851 CA MET A 217 58.394 8.785 35.636 1.00 22.41 C
ATOM 1852 C MET A 217 59.572 7.942 35.992 1.00 27.54 C
ATOM 1853 O MET A 217 59.579 6.752 35.710 1.00 20.86 O
ATOM 1854 CB MET A 217 58.637 9.592 34.345 1.00 21.24 C

ATOM	1855	CG	MET	A	217	59.478	8.918	33.287	1.00	16.37	C
ATOM	1856	SD	MET	A	217	58.962	7.412	32.473	1.00	30.51	S
ATOM	1857	CE	MET	A	217	57.465	7.608	32.391	1.00	19.57	C
ATOM	1858	N	ALA	A	218	60.561	8.562	36.623	1.00	19.09	N
ATOM	1859	CA	ALA	A	218	61.774	7.841	37.002	1.00	13.65	C
ATOM	1860	C	ALA	A	218	61.436	6.778	38.028	1.00	22.61	C
ATOM	1861	O	ALA	A	218	61.934	5.670	37.967	1.00	19.36	O
ATOM	1862	CB	ALA	A	218	62.809	8.780	37.579	1.00	12.23	C
ATOM	1863	N	ALA	A	219	60.605	7.109	39.000	1.00	19.34	N
ATOM	1864	CA	ALA	A	219	60.310	6.105	40.023	1.00	18.01	C
ATOM	1865	C	ALA	A	219	59.630	4.901	39.413	1.00	23.57	C
ATOM	1866	O	ALA	A	219	59.781	3.777	39.898	1.00	22.71	O
ATOM	1867	CB	ALA	A	219	59.387	6.678	41.083	1.00	10.11	C
ATOM	1868	N	ALA	A	220	58.753	5.174	38.454	1.00	18.99	N
ATOM	1869	CA	ALA	A	220	57.905	4.158	37.855	1.00	14.12	C
ATOM	1870	C	ALA	A	220	58.753	3.213	37.034	1.00	25.33	C
ATOM	1871	O	ALA	A	220	58.584	2.006	37.114	1.00	20.63	O
ATOM	1872	CB	ALA	A	220	56.796	4.798	37.023	1.00	8.53	C
ATOM	1873	N	SER	A	221	59.770	3.772	36.379	1.00	23.92	N
ATOM	1874	CA	SER	A	221	60.702	3.011	35.556	1.00	18.38	C
ATOM	1875	C	SER	A	221	61.537	1.989	36.353	1.00	20.90	C
ATOM	1876	O	SER	A	221	61.683	0.799	35.983	1.00	19.84	O
ATOM	1877	CB	SER	A	221	61.604	3.985	34.804	1.00	10.67	C
ATOM	1878	OG	SER	A	221	60.847	4.744	33.867	1.00	15.61	O
ATOM	1879	N	ILE	A	222	62.083	2.476	37.463	1.00	18.12	N
ATOM	1880	CA	ILE	A	222	62.866	1.644	38.381	1.00	21.56	C
ATOM	1881	C	ILE	A	222	62.020	0.554	39.068	1.00	29.10	C
ATOM	1882	O	ILE	A	222	62.504	-0.566	39.307	1.00	19.03	O
ATOM	1883	CB	ILE	A	222	63.467	2.516	39.432	1.00	24.56	C
ATOM	1884	CG1	ILE	A	222	64.465	3.473	38.765	1.00	32.13	C
ATOM	1885	CG2	ILE	A	222	64.129	1.671	40.500	1.00	28.26	C
ATOM	1886	CD1	ILE	A	222	64.973	4.585	39.649	1.00	15.61	C
ATOM	1887	N	HIS	A	223	60.772	0.907	39.384	1.00	19.34	N
ATOM	1888	CA	HIS	A	223	59.829	-0.031	39.996	1.00	20.46	C
ATOM	1889	C	HIS	A	223	59.599	-1.097	38.964	1.00	24.82	C
ATOM	1890	O	HIS	A	223	59.723	-2.283	39.270	1.00	24.66	O
ATOM	1891	CB	HIS	A	223	58.465	0.637	40.359	1.00	19.53	C
ATOM	1892	CG	HIS	A	223	57.373	-0.333	40.759	1.00	28.64	C
ATOM	1893	ND1	HIS	A	223	57.021	-0.564	42.082	1.00	24.16	N
ATOM	1894	CD2	HIS	A	223	56.497	-1.062	40.004	1.00	30.39	C
ATOM	1895	CE1	HIS	A	223	55.983	-1.399	42.112	1.00	30.39	C
ATOM	1896	NE2	HIS	A	223	55.652	-1.727	40.869	1.00	28.13	N
ATOM	1897	N	VAL	A	224	59.354	-0.684	37.725	1.00	22.06	N
ATOM	1898	CA	VAL	A	224	59.111	-1.657	36.652	1.00	19.15	C
ATOM	1899	C	VAL	A	224	60.350	-2.490	36.333	1.00	25.89	C

ATOM 1900 O VAL A 224 60.282 -3.709 36.250 1.00 22.37 O
ATOM 1901 CB VAL A 224 58.559 -1.022 35.377 1.00 22.59 C
ATOM 1902 CG1 VAL A 224 58.512 -2.050 34.231 1.00 22.61 C
ATOM 1903 CG2 VAL A 224 57.161 -0.491 35.650 1.00 23.44 C
ATOM 1904 N MET A 225 61.499 -1.838 36.255 1.00 27.83 N
ATOM 1905 CA MET A 225 62.710 -2.577 36.004 1.00 23.69 C
ATOM 1906 C MET A 225 62.896 -3.678 37.071 1.00 31.95 C
ATOM 1907 O MET A 225 63.290 -4.805 36.785 1.00 24.33 O
ATOM 1908 CB MET A 225 63.902 -1.604 36.056 1.00 21.34 C
ATOM 1909 CG MET A 225 65.295 -2.296 35.999 1.00 17.83 C
ATOM 1910 SD MET A 225 65.750 -2.958 34.306 1.00 23.33 S
ATOM 1911 CE MET A 225 67.080 -1.896 33.785 1.00 16.46 C
ATOM 1912 N GLU A 226 62.644 -3.319 38.316 1.00 19.54 N
ATOM 1913 CA GLU A 226 62.988 -4.161 39.428 1.00 21.58 C
ATOM 1914 C GLU A 226 61.999 -5.200 39.918 1.00 30.77 C
ATOM 1915 O GLU A 226 62.308 -6.012 40.780 1.00 29.39 O
ATOM 1916 CB GLU A 226 63.613 -3.323 40.547 1.00 20.47 C
ATOM 1917 CG GLU A 226 64.937 -2.673 40.122 1.00 23.03 C
ATOM 1918 CD GLU A 226 65.504 -1.809 41.208 1.00 32.62 C
ATOM 1919 OE1 GLU A 226 64.721 -1.455 42.122 1.00 26.12 O
ATOM 1920 OE2 GLU A 226 66.711 -1.479 41.152 1.00 17.67 O
ATOM 1921 N LEU A 227 60.837 -5.248 39.295 1.00 34.11 N
ATOM 1922 CA LEU A 227 59.883 -6.296 39.642 1.00 35.26 C
ATOM 1923 C LEU A 227 60.537 -7.644 39.320 1.00 27.91 C
ATOM 1924 O LEU A 227 61.291 -7.766 38.340 1.00 19.89 O
ATOM 1925 CB LEU A 227 58.693 -6.236 38.678 1.00 36.48 C
ATOM 1926 CG LEU A 227 57.381 -5.569 38.955 1.00 40.30 C
ATOM 1927 CD1 LEU A 227 57.697 -4.194 39.382 1.00 42.04 C
ATOM 1928 CD2 LEU A 227 56.610 -5.577 37.647 1.00 46.21 C
ATOM 1929 N ALA A 228 60.026 -8.688 39.955 1.00 27.15 N
ATOM 1930 CA ALA A 228 60.425 -10.051 39.616 1.00 25.26 C
ATOM 1931 C ALA A 228 59.801 -10.435 38.279 1.00 27.93 C
ATOM 1932 O ALA A 228 58.624 -10.093 37.934 1.00 31.26 O
ATOM 1933 CB ALA A 228 60.003 -11.052 40.703 1.00 22.05 C
ATOM 1934 N HIS A 229 60.624 -11.160 37.539 1.00 27.05 N
ATOM 1935 CA HIS A 229 60.275 -11.605 36.222 1.00 24.42 C
ATOM 1936 C HIS A 229 58.905 -12.260 36.184 1.00 21.74 C
ATOM 1937 O HIS A 229 58.015 -11.851 35.398 1.00 22.22 O
ATOM 1938 CB HIS A 229 61.351 -12.520 35.698 1.00 17.71 C
ATOM 1939 CG HIS A 229 61.284 -12.701 34.220 1.00 27.24 C
ATOM 1940 ND1 HIS A 229 61.060 -11.650 33.350 1.00 34.38 N
ATOM 1941 CD2 HIS A 229 61.292 -13.821 33.465 1.00 31.45 C
ATOM 1942 CE1 HIS A 229 60.992 -12.113 32.115 1.00 30.50 C
ATOM 1943 NE2 HIS A 229 61.124 -13.427 32.159 1.00 35.23 N
ATOM 1944 N GLU A 230 58.681 -13.161 37.140 1.00 20.24 N

ATOM 1945 CA GLU A 230 57.425 -13.895 37.209 1.00 29.41 C
ATOM 1946 C GLU A 230 56.181 -13.051 37.341 1.00 22.20 C
ATOM 1947 O GLU A 230 55.159 -13.359 36.679 1.00 17.78 O
ATOM 1948 CB GLU A 230 57.464 -14.997 38.274 1.00 38.51 C
ATOM 1949 CG GLU A 230 58.085 -14.582 39.567 1.00 63.09 C
ATOM 1950 CD GLU A 230 57.036 -14.473 40.661 1.00100.00 C
ATOM 1951 OE1 GLU A 230 55.859 -14.872 40.400 1.00100.00 O
ATOM 1952 OE2 GLU A 230 57.409 -14.003 41.768 1.00 81.48 O
ATOM 1953 N VAL A 231 56.272 -12.004 38.182 1.00 16.53 N
ATOM 1954 CA VAL A 231 55.202 -11.029 38.356 1.00 20.23 C
ATOM 1955 C VAL A 231 55.009 -10.164 37.102 1.00 24.45 C
ATOM 1956 O VAL A 231 53.864 -9.834 36.705 1.00 21.00 O
ATOM 1957 CB VAL A 231 55.541 -10.057 39.426 1.00 28.61 C
ATOM 1958 CG1 VAL A 231 54.362 -9.098 39.610 1.00 29.78 C
ATOM 1959 CG2 VAL A 231 55.881 -10.757 40.677 1.00 28.96 C
ATOM 1960 N TRP A 232 56.133 -9.798 36.486 1.00 17.17 N
ATOM 1961 CA TRP A 232 56.052 -9.044 35.262 1.00 21.52 C
ATOM 1962 C TRP A 232 55.388 -9.844 34.156 1.00 20.53 C
ATOM 1963 O TRP A 232 54.588 -9.306 33.380 1.00 24.31 O
ATOM 1964 CB TRP A 232 57.438 -8.644 34.801 1.00 29.88 C
ATOM 1965 CG TRP A 232 57.430 -7.843 33.500 1.00 27.65 C
ATOM 1966 CD1 TRP A 232 57.184 -6.464 33.356 1.00 25.42 C
ATOM 1967 CD2 TRP A 232 57.714 -8.336 32.169 1.00 27.75 C
ATOM 1968 NE1 TRP A 232 57.325 -6.095 32.033 1.00 22.53 N
ATOM 1969 CE2 TRP A 232 57.655 -7.203 31.279 1.00 25.11 C
ATOM 1970 CE3 TRP A 232 58.037 -9.603 31.640 1.00 22.72 C
ATOM 1971 CZ2 TRP A 232 57.917 -7.316 29.879 1.00 17.23 C
ATOM 1972 CZ3 TRP A 232 58.238 -9.720 30.223 1.00 25.97 C
ATOM 1973 CH2 TRP A 232 58.154 -8.581 29.368 1.00 22.07 C
ATOM 1974 N LEU A 233 55.749 -11.121 34.018 1.00 23.80 N
ATOM 1975 CA LEU A 233 55.141 -11.949 32.937 1.00 24.78 C
ATOM 1976 C LEU A 233 53.652 -12.118 33.122 1.00 24.51 C
ATOM 1977 O LEU A 233 52.865 -12.075 32.163 1.00 28.50 O
ATOM 1978 CB LEU A 233 55.765 -13.348 32.820 1.00 26.20 C
ATOM 1979 CG LEU A 233 57.250 -13.505 32.503 1.00 19.39 C
ATOM 1980 CD1 LEU A 233 57.745 -14.850 33.023 1.00 19.90 C
ATOM 1981 CD2 LEU A 233 57.561 -13.287 31.017 1.00 16.01 C
ATOM 1982 N GLU A 234 53.298 -12.343 34.372 1.00 25.45 N
ATOM 1983 CA GLU A 234 51.929 -12.523 34.822 1.00 30.04 C
ATOM 1984 C GLU A 234 51.128 -11.319 34.367 1.00 35.69 C
ATOM 1985 O GLU A 234 49.926 -11.390 34.052 1.00 28.25 O
ATOM 1986 CB GLU A 234 52.007 -12.468 36.344 1.00 37.30 C
ATOM 1987 CG GLU A 234 50.908 -13.133 37.118 1.00 45.39 C
ATOM 1988 CD GLU A 234 51.112 -12.881 38.601 1.00100.00 C
ATOM 1989 OE1 GLU A 234 52.240 -13.137 39.104 1.00 99.09 O

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ATOM   1990  OE2 GLU A 234      50.211 -12.257  39.211  1.00100.00           O
ATOM   1991  N   ASN A 235      51.802 -10.184  34.364  1.00 25.04           N
ATOM   1992  CA  ASN A 235      51.109  -8.986  33.992  1.00 26.17           C
ATOM   1993  C   ASN A 235      51.280  -8.494  32.571  1.00 30.46           C
ATOM   1994  O   ASN A 235      50.824  -7.393  32.259  1.00 22.90           O
ATOM   1995  CB  ASN A 235      51.427  -7.895  34.981  1.00 29.23           C
ATOM   1996  CG  ASN A 235      50.878  -8.197  36.342  1.00 39.27           C
ATOM   1997  OD1 ASN A 235      49.722  -7.882  36.628  1.00 29.06           O
ATOM   1998  ND2 ASN A 235      51.653  -8.934  37.140  1.00 40.22           N
ATOM   1999  N   THR A 236      51.935  -9.268  31.708  1.00 20.97           N
ATOM   2000  CA  THR A 236      52.108  -8.795  30.344  1.00 22.30           C
ATOM   2001  C   THR A 236      51.867  -9.943  29.419  1.00 29.74           C
ATOM   2002  O   THR A 236      51.551 -11.033  29.895  1.00 21.23           O
ATOM   2003  CB  THR A 236      53.545  -8.306  30.161  1.00 22.73           C
ATOM   2004  OG1 THR A 236      54.422  -9.325  30.636  1.00 21.23           O
ATOM   2005  CG2 THR A 236      53.801  -7.048  31.041  1.00 19.69           C
ATOM   2006  N   GLN A 237      52.003  -9.699  28.109  1.00 22.23           N
ATOM   2007  CA  GLN A 237      52.097 -10.783  27.122  1.00 16.69           C
ATOM   2008  C   GLN A 237      53.335 -10.507  26.331  1.00 21.02           C
ATOM   2009  O   GLN A 237      53.729  -9.362  26.204  1.00 22.19           O
ATOM   2010  CB  GLN A 237      50.913 -10.999  26.189  1.00  8.23           C
ATOM   2011  CG  GLN A 237      49.639 -11.096  26.904  1.00 21.04           C
ATOM   2012  CD  GLN A 237      48.907  -9.862  26.606  1.00 62.07           C
ATOM   2013  OE1 GLN A 237      48.437  -9.712  25.460  1.00 59.32           O
ATOM   2014  NE2 GLN A 237      49.220  -8.847  27.388  1.00 37.82           N
ATOM   2015  N   PRO A 238      54.002 -11.579  25.917  1.00 28.76           N
ATOM   2016  CA  PRO A 238      55.275 -11.438  25.246  1.00 30.28           C
ATOM   2017  C   PRO A 238      55.194 -10.643  23.958  1.00 29.08           C
ATOM   2018  O   PRO A 238      56.181 -10.029  23.600  1.00 15.95           O
ATOM   2019  CB  PRO A 238      55.733 -12.879  25.011  1.00 22.54           C
ATOM   2020  CG  PRO A 238      54.898 -13.710  25.886  1.00 18.92           C
ATOM   2021  CD  PRO A 238      53.626 -12.998  26.068  1.00 11.75           C
ATOM   2022  N   MET A 239      54.041 -10.635  23.286  1.00 17.26           N
ATOM   2023  CA  MET A 239      53.924  -9.807  22.104  1.00 17.85           C
ATOM   2024  C   MET A 239      53.109  -8.509  22.362  1.00 18.63           C
ATOM   2025  O   MET A 239      52.792  -7.741  21.419  1.00 16.82           O
ATOM   2026  CB  MET A 239      53.460 -10.588  20.881  1.00 15.22           C
ATOM   2027  CG  MET A 239      54.536 -11.534  20.261  1.00 12.90           C
ATOM   2028  SD  MET A 239      53.994 -12.534  18.808  1.00 17.49           S
ATOM   2029  CE  MET A 239      54.350 -11.357  17.422  1.00 13.12           C
ATOM   2030  N   LEU A 240      52.847  -8.252  23.646  1.00 18.55           N
ATOM   2031  CA  LEU A 240      52.159  -7.037  24.131  1.00 16.68           C
ATOM   2032  C   LEU A 240      52.774  -6.733  25.493  1.00 11.82           C
ATOM   2033  O   LEU A 240      52.124  -6.803  26.549  1.00 13.84           O
ATOM   2034  CB  LEU A 240      50.645  -7.249  24.240  1.00 16.91           C
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ATOM	2035	CG	LEU A 240	49.646	-6.120	23.852	1.00	22.29	C
ATOM	2036	CD1	LEU A 240	48.968	-5.488	25.033	1.00	25.51	C
ATOM	2037	CD2	LEU A 240	50.070	-5.059	22.815	1.00	28.07	C
ATOM	2038	N	SER A 241	54.076	-6.467	25.456	1.00	13.09	N
ATOM	2039	CA	SER A 241	54.842	-6.315	26.682	1.00	24.20	C
ATOM	2040	C	SER A 241	54.947	-4.938	27.377	1.00	30.52	C
ATOM	2041	O	SER A 241	55.363	-4.854	28.547	1.00	17.02	O
ATOM	2042	CB	SER A 241	56.247	-6.900	26.495	1.00	14.04	C
ATOM	2043	OG	SER A 241	57.062	-6.144	25.598	1.00	13.95	O
ATOM	2044	N	HIS A 242	54.661	-3.861	26.659	1.00	17.87	N
ATOM	2045	CA	HIS A 242	54.894	-2.548	27.221	1.00	13.55	C
ATOM	2046	C	HIS A 242	53.990	-2.254	28.373	1.00	13.70	C
ATOM	2047	O	HIS A 242	52.974	-2.885	28.539	1.00	13.29	O
ATOM	2048	CB	HIS A 242	54.826	-1.430	26.130	1.00	16.05	C
ATOM	2049	CG	HIS A 242	53.595	-1.504	25.272	1.00	18.88	C
ATOM	2050	ND1	HIS A 242	52.591	-0.553	25.326	1.00	23.24	N
ATOM	2051	CD2	HIS A 242	53.165	-2.461	24.413	1.00	13.19	C
ATOM	2052	CE1	HIS A 242	51.629	-0.887	24.483	1.00	17.44	C
ATOM	2053	NE2	HIS A 242	51.962	-2.031	23.901	1.00	19.54	N
ATOM	2054	N	ILE A 243	54.310	-1.203	29.095	1.00	15.84	N
ATOM	2055	CA	ILE A 243	53.492	-0.809	30.192	1.00	19.10	C
ATOM	2056	C	ILE A 243	53.336	0.714	30.191	1.00	23.23	C
ATOM	2057	O	ILE A 243	54.312	1.406	30.385	1.00	12.10	O
ATOM	2058	CB	ILE A 243	54.166	-1.273	31.482	1.00	24.62	C
ATOM	2059	CG1	ILE A 243	54.014	-2.783	31.576	1.00	25.60	C
ATOM	2060	CG2	ILE A 243	53.497	-0.665	32.735	1.00	17.37	C
ATOM	2061	CD1	ILE A 243	54.725	-3.365	32.714	1.00	14.82	C
ATOM	2062	N	ASN A 244	52.112	1.217	30.013	1.00	16.43	N
ATOM	2063	CA	ASN A 244	51.824	2.689	30.038	1.00	18.99	C
ATOM	2064	C	ASN A 244	52.252	3.292	31.348	1.00	18.83	C
ATOM	2065	O	ASN A 244	51.965	2.727	32.405	1.00	19.58	O
ATOM	2066	CB	ASN A 244	50.304	2.987	29.910	1.00	15.67	C
ATOM	2067	CG	ASN A 244	49.768	2.702	28.517	1.00	14.57	C
ATOM	2068	OD1	ASN A 244	50.546	2.583	27.580	1.00	13.64	O
ATOM	2069	ND2	ASN A 244	48.443	2.491	28.393	1.00	10.16	N
ATOM	2070	N	VAL A 245	52.800	4.499	31.326	1.00	13.50	N
ATOM	2071	CA	VAL A 245	53.159	5.134	32.602	1.00	13.49	C
ATOM	2072	C	VAL A 245	52.528	6.566	32.644	1.00	16.25	C
ATOM	2073	O	VAL A 245	52.786	7.405	31.770	1.00	15.20	O
ATOM	2074	CB	VAL A 245	54.754	5.163	32.810	1.00	21.07	C
ATOM	2075	CG1	VAL A 245	55.154	6.085	33.937	1.00	15.08	C
ATOM	2076	CG2	VAL A 245	55.280	3.817	33.143	1.00	15.82	C
ATOM	2077	N	GLY A 246	51.696	6.843	33.649	1.00	14.03	N
ATOM	2078	CA	GLY A 246	51.027	8.136	33.707	1.00	16.87	C
ATOM	2079	C	GLY A 246	50.146	8.203	34.939	1.00	26.95	C

ATOM	2080	O	GLY A 246	50.323	7.401	35.850	1.00	23.04		O
ATOM	2081	N	THR A 247	49.207	9.161	34.963	1.00	21.44		N
ATOM	2082	CA	THR A 247	48.232	9.276	36.063	1.00	21.39		C
ATOM	2083	C	THR A 247	46.868	8.677	35.673	1.00	24.08		C
ATOM	2084	O	THR A 247	46.069	8.306	36.508	1.00	21.03		O
ATOM	2085	CB	THR A 247	47.988	10.730	36.404	1.00	22.24		C
ATOM	2086	OG1	THR A 247	47.409	11.389	35.265	1.00	18.62		O
ATOM	2087	CG2	THR A 247	49.275	11.378	36.724	1.00	18.99		C
ATOM	2088	N	GLY A 248	46.583	8.651	34.384	1.00	24.95		N
ATOM	2089	CA	GLY A 248	45.319	8.143	33.924	1.00	22.61		C
ATOM	2090	C	GLY A 248	44.223	9.160	34.226	1.00	21.42		C
ATOM	2091	O	GLY A 248	43.059	8.866	34.137	1.00	25.70		O
ATOM	2092	N	VAL A 249	44.615	10.386	34.521	1.00	30.72		N
ATOM	2093	CA	VAL A 249	43.673	11.464	34.827	1.00	26.09		C
ATOM	2094	C	VAL A 249	43.747	12.596	33.786	1.00	32.70		C
ATOM	2095	O	VAL A 249	44.853	13.006	33.387	1.00	26.92		O
ATOM	2096	CB	VAL A 249	44.020	12.085	36.214	1.00	38.59		C
ATOM	2097	CG1	VAL A 249	43.225	13.324	36.470	1.00	36.11		C
ATOM	2098	CG2	VAL A 249	43.782	11.083	37.306	1.00	41.30		C
ATOM	2099	N	ASP A 250	42.581	13.125	33.397	1.00	27.95		N
ATOM	2100	CA	ASP A 250	42.488	14.232	32.439	1.00	20.64		C
ATOM	2101	C	ASP A 250	42.611	15.581	33.155	1.00	27.63		C
ATOM	2102	O	ASP A 250	42.188	15.783	34.308	1.00	26.23		O
ATOM	2103	CB	ASP A 250	41.075	14.302	31.827	1.00	23.89		C
ATOM	2104	CG	ASP A 250	40.768	13.180	30.850	1.00	39.52		C
ATOM	2105	OD1	ASP A 250	41.283	13.184	29.688	1.00	39.96		O
ATOM	2106	OD2	ASP A 250	39.767	12.501	31.153	1.00	45.34		O
ATOM	2107	N	CYS A 251	43.029	16.566	32.388	1.00	20.12		N
ATOM	2108	CA	CYS A 251	42.962	17.906	32.851	1.00	27.20		C
ATOM	2109	C	CYS A 251	42.918	18.779	31.577	1.00	26.47		C
ATOM	2110	O	CYS A 251	43.699	18.560	30.633	1.00	19.45		O
ATOM	2111	CB	CYS A 251	44.148	18.157	33.778	1.00	34.86		C
ATOM	2112	SG	CYS A 251	45.129	19.619	33.453	1.00	29.47		S
ATOM	2113	N	THR A 252	41.932	19.673	31.494	1.00	14.85		N
ATOM	2114	CA	THR A 252	41.834	20.588	30.335	1.00	21.21		C
ATOM	2115	C	THR A 252	42.999	21.592	30.236	1.00	20.53		C
ATOM	2116	O	THR A 252	43.657	21.926	31.249	1.00	15.24		O
ATOM	2117	CB	THR A 252	40.506	21.407	30.329	1.00	32.08		C
ATOM	2118	OG1	THR A 252	40.460	22.304	31.447	1.00	19.26		O
ATOM	2119	CG2	THR A 252	39.309	20.495	30.372	1.00	13.91		C
ATOM	2120	N	ILE A 253	43.228	22.095	29.024	1.00	14.81		N
ATOM	2121	CA	ILE A 253	44.264	23.118	28.812	1.00	16.90		C
ATOM	2122	C	ILE A 253	43.934	24.383	29.627	1.00	23.41		C
ATOM	2123	O	ILE A 253	44.834	25.012	30.247	1.00	15.27		O
ATOM	2124	CB	ILE A 253	44.404	23.452	27.302	1.00	24.05		C

ATOM 2125 CG1 ILE A 253 44.862 22.200 26.561 1.00 27.33 C
ATOM 2126 CG2 ILE A 253 45.473 24.479 27.077 1.00 9.22 C
ATOM 2127 CD1 ILE A 253 45.662 21.276 27.452 1.00 49.56 C
ATOM 2128 N ARG A 254 42.637 24.709 29.707 1.00 19.56 N
ATOM 2129 CA ARG A 254 42.228 25.865 30.522 1.00 19.41 C
ATOM 2130 C ARG A 254 42.712 25.713 31.970 1.00 18.10 C
ATOM 2131 O ARG A 254 43.311 26.616 32.515 1.00 13.89 O
ATOM 2132 CB ARG A 254 40.704 26.101 30.480 1.00 15.98 C
ATOM 2133 CG ARG A 254 40.282 27.378 31.255 1.00 9.96 C
ATOM 2134 CD ARG A 254 38.809 27.702 31.218 1.00 24.79 C
ATOM 2135 NE ARG A 254 38.498 28.414 29.997 1.00 29.42 N
ATOM 2136 CZ ARG A 254 38.693 29.723 29.794 1.00 59.85 C
ATOM 2137 NH1 ARG A 254 39.194 30.527 30.732 1.00 42.58 N
ATOM 2138 NH2 ARG A 254 38.377 30.245 28.620 1.00 18.44 N
ATOM 2139 N ASP A 255 42.406 24.564 32.586 1.00 20.22 N
ATOM 2140 CA ASP A 255 42.795 24.205 33.974 1.00 16.48 C
ATOM 2141 C ASP A 255 44.321 24.372 34.069 1.00 22.43 C
ATOM 2142 O ASP A 255 44.868 24.897 35.060 1.00 18.53 O
ATOM 2143 CB ASP A 255 42.478 22.686 34.157 1.00 19.17 C
ATOM 2144 CG ASP A 255 42.144 22.246 35.610 1.00 47.08 C
ATOM 2145 OD1 ASP A 255 41.780 23.090 36.429 1.00 49.66 O
ATOM 2146 OD2 ASP A 255 42.020 21.016 35.880 1.00 48.12 O
ATOM 2147 N LEU A 256 45.014 23.809 33.078 1.00 15.98 N
ATOM 2148 CA LEU A 256 46.465 23.844 33.069 1.00 21.76 C
ATOM 2149 C LEU A 256 47.020 25.275 33.076 1.00 16.79 C
ATOM 2150 O LEU A 256 47.825 25.697 33.946 1.00 15.24 O
ATOM 2151 CB LEU A 256 46.967 23.056 31.859 1.00 23.33 C
ATOM 2152 CG LEU A 256 48.491 23.100 31.765 1.00 26.80 C
ATOM 2153 CD1 LEU A 256 49.171 22.334 32.984 1.00 17.13 C
ATOM 2154 CD2 LEU A 256 49.040 22.724 30.346 1.00 15.42 C
ATOM 2155 N ALA A 257 46.520 26.048 32.140 1.00 13.77 N
ATOM 2156 CA ALA A 257 46.938 27.436 32.025 1.00 12.70 C
ATOM 2157 C ALA A 257 46.656 28.237 33.267 1.00 10.73 C
ATOM 2158 O ALA A 257 47.451 29.073 33.672 1.00 20.33 O
ATOM 2159 CB ALA A 257 46.208 28.073 30.834 1.00 13.34 C
ATOM 2160 N GLN A 258 45.470 28.080 33.835 1.00 12.40 N
ATOM 2161 CA GLN A 258 45.102 28.911 34.981 1.00 8.39 C
ATOM 2162 C GLN A 258 45.879 28.480 36.166 1.00 13.48 C
ATOM 2163 O GLN A 258 46.178 29.281 37.029 1.00 22.96 O
ATOM 2164 CB GLN A 258 43.614 28.761 35.305 1.00 16.12 C
ATOM 2165 CG GLN A 258 42.674 29.096 34.130 1.00 30.19 C
ATOM 2166 CD GLN A 258 42.574 30.585 33.781 1.00 37.29 C
ATOM 2167 OE1 GLN A 258 42.911 31.471 34.610 1.00 21.24 O
ATOM 2168 NE2 GLN A 258 42.021 30.876 32.572 1.00 15.94 N
ATOM 2169 N THR A 259 46.179 27.182 36.232 1.00 16.21 N

ATOM 2170 CA THR A 259 46.982 26.678 37.336 1.00 16.85 C
ATOM 2171 C THR A 259 48.410 27.186 37.233 1.00 20.56 C
ATOM 2172 O THR A 259 49.002 27.621 38.214 1.00 21.44 O
ATOM 2173 CB THR A 259 47.066 25.192 37.361 1.00 27.56 C
ATOM 2174 OG1 THR A 259 45.752 24.620 37.509 1.00 20.92 O
ATOM 2175 CG2 THR A 259 47.936 24.796 38.545 1.00 12.85 C
ATOM 2176 N ILE A 260 48.952 27.170 36.028 1.00 19.96 N
ATOM 2177 CA ILE A 260 50.292 27.704 35.839 1.00 23.01 C
ATOM 2178 C ILE A 260 50.313 29.180 36.225 1.00 31.73 C
ATOM 2179 O ILE A 260 51.211 29.627 36.993 1.00 25.90 O
ATOM 2180 CB ILE A 260 50.835 27.456 34.390 1.00 22.46 C
ATOM 2181 CG1 ILE A 260 51.153 25.940 34.232 1.00 24.12 C
ATOM 2182 CG2 ILE A 260 52.099 28.361 34.106 1.00 13.47 C
ATOM 2183 CD1 ILE A 260 51.501 25.443 32.810 1.00 12.58 C
ATOM 2184 N ALA A 261 49.280 29.910 35.764 1.00 15.35 N
ATOM 2185 CA ALA A 261 49.177 31.355 36.048 1.00 16.00 C
ATOM 2186 C ALA A 261 49.316 31.604 37.550 1.00 20.58 C
ATOM 2187 O ALA A 261 50.104 32.443 37.987 1.00 16.09 O
ATOM 2188 CB ALA A 261 47.832 31.958 35.487 1.00 13.65 C
ATOM 2189 N LYS A 262 48.551 30.843 38.323 1.00 11.50 N
ATOM 2190 CA LYS A 262 48.578 30.905 39.770 1.00 10.13 C
ATOM 2191 C LYS A 262 49.968 30.460 40.296 1.00 28.08 C
ATOM 2192 O LYS A 262 50.503 31.084 41.205 1.00 29.37 O
ATOM 2193 CB LYS A 262 47.453 30.032 40.335 1.00 12.50 C
ATOM 2194 CG LYS A 262 47.332 29.962 41.888 1.00 16.51 C
ATOM 2195 CD LYS A 262 46.092 29.092 42.371 1.00 46.61 C
ATOM 2196 CE LYS A 262 46.344 27.555 42.661 1.00 99.70 C
ATOM 2197 NZ LYS A 262 45.157 26.703 43.200 1.00 36.59 N
ATOM 2198 N VAL A 263 50.589 29.443 39.705 1.00 17.44 N
ATOM 2199 CA VAL A 263 51.915 29.039 40.171 1.00 18.72 C
ATOM 2200 C VAL A 263 52.997 30.170 39.997 1.00 32.12 C
ATOM 2201 O VAL A 263 53.871 30.412 40.834 1.00 21.18 O
ATOM 2202 CB VAL A 263 52.389 27.709 39.476 1.00 16.35 C
ATOM 2203 CG1 VAL A 263 53.920 27.518 39.647 1.00 11.83 C
ATOM 2204 CG2 VAL A 263 51.646 26.522 40.093 1.00 14.99 C
ATOM 2205 N VAL A 264 52.913 30.899 38.909 1.00 21.75 N
ATOM 2206 CA VAL A 264 53.917 31.877 38.653 1.00 19.81 C
ATOM 2207 C VAL A 264 53.719 33.208 39.377 1.00 35.79 C
ATOM 2208 O VAL A 264 54.632 34.032 39.482 1.00 28.99 O
ATOM 2209 CB VAL A 264 54.059 32.014 37.175 1.00 24.27 C
ATOM 2210 CG1 VAL A 264 54.728 33.269 36.822 1.00 33.58 C
ATOM 2211 CG2 VAL A 264 54.840 30.808 36.674 1.00 23.01 C
ATOM 2212 N GLY A 265 52.550 33.378 39.969 1.00 25.30 N
ATOM 2213 CA GLY A 265 52.241 34.620 40.636 1.00 24.14 C
ATOM 2214 C GLY A 265 51.730 35.694 39.632 1.00 35.03 C

ATOM	2215	O	GLY	A	265	51.773	36.911	39.962	1.00	33.71	O
ATOM	2216	N	TYR	A	266	51.294	35.257	38.428	1.00	26.25	N
ATOM	2217	CA	TYR	A	266	50.698	36.151	37.373	1.00	26.55	C
ATOM	2218	C	TYR	A	266	49.364	36.745	37.818	1.00	31.01	C
ATOM	2219	O	TYR	A	266	48.532	36.067	38.456	1.00	27.99	O
ATOM	2220	CB	TYR	A	266	50.501	35.463	36.008	1.00	24.31	C
ATOM	2221	CG	TYR	A	266	49.994	36.381	34.884	1.00	28.64	C
ATOM	2222	CD1	TYR	A	266	50.670	37.582	34.542	1.00	35.05	C
ATOM	2223	CD2	TYR	A	266	48.860	36.038	34.118	1.00	22.60	C
ATOM	2224	CE1	TYR	A	266	50.212	38.434	33.472	1.00	20.73	C
ATOM	2225	CE2	TYR	A	266	48.428	36.859	33.012	1.00	20.91	C
ATOM	2226	CZ	TYR	A	266	49.088	38.062	32.735	1.00	23.85	C
ATOM	2227	OH	TYR	A	266	48.622	38.851	31.710	1.00	33.40	O
ATOM	2228	N	LYS	A	267	49.217	38.043	37.604	1.00	25.72	N
ATOM	2229	CA	LYS	A	267	47.988	38.697	38.009	1.00	30.77	C
ATOM	2230	C	LYS	A	267	47.217	39.280	36.798	1.00	28.85	C
ATOM	2231	O	LYS	A	267	46.179	39.894	36.949	1.00	31.17	O
ATOM	2232	CB	LYS	A	267	48.279	39.741	39.092	1.00	27.13	C
ATOM	2233	CG	LYS	A	267	48.728	39.128	40.403	1.00	23.18	C
ATOM	2234	CD	LYS	A	267	48.420	40.096	41.562	1.00	30.98	C
ATOM	2235	CE	LYS	A	267	47.933	39.358	42.820	1.00	48.52	C
ATOM	2236	NZ	LYS	A	267	47.005	38.208	42.505	1.00	100.00	N
ATOM	2237	N	GLY	A	268	47.716	39.054	35.594	1.00	22.67	N
ATOM	2238	CA	GLY	A	268	47.019	39.518	34.394	1.00	21.38	C
ATOM	2239	C	GLY	A	268	45.856	38.568	34.085	1.00	31.03	C
ATOM	2240	O	GLY	A	268	45.455	37.728	34.911	1.00	19.71	O
ATOM	2241	N	ARG	A	269	45.387	38.645	32.849	1.00	30.40	N
ATOM	2242	CA	ARG	A	269	44.263	37.846	32.399	1.00	26.47	C
ATOM	2243	C	ARG	A	269	44.680	36.705	31.489	1.00	22.35	C
ATOM	2244	O	ARG	A	269	45.378	36.926	30.524	1.00	22.75	O
ATOM	2245	CB	ARG	A	269	43.297	38.753	31.626	1.00	22.65	C
ATOM	2246	CG	ARG	A	269	42.201	39.390	32.463	1.00	24.21	C
ATOM	2247	CD	ARG	A	269	40.936	39.465	31.568	1.00	83.45	C
ATOM	2248	NE	ARG	A	269	40.113	40.676	31.762	1.00	100.00	N
ATOM	2249	CZ	ARG	A	269	38.808	40.751	31.431	1.00	100.00	C
ATOM	2250	NH1	ARG	A	269	38.201	39.691	30.921	1.00	99.93	N
ATOM	2251	NH2	ARG	A	269	38.094	41.865	31.663	1.00	100.00	N
ATOM	2252	N	VAL	A	270	44.195	35.494	31.758	1.00	19.87	N
ATOM	2253	CA	VAL	A	270	44.468	34.389	30.856	1.00	24.82	C
ATOM	2254	C	VAL	A	270	43.319	34.456	29.824	1.00	22.51	C
ATOM	2255	O	VAL	A	270	42.145	34.501	30.181	1.00	25.79	O
ATOM	2256	CB	VAL	A	270	44.436	32.979	31.571	1.00	24.03	C
ATOM	2257	CG1	VAL	A	270	44.576	31.861	30.533	1.00	20.72	C
ATOM	2258	CG2	VAL	A	270	45.506	32.849	32.639	1.00	11.27	C
ATOM	2259	N	VAL	A	271	43.660	34.409	28.554	1.00	25.18	N

ATOM 2260 CA VAL A 271 42.666 34.492 27.487 1.00 28.32 C
ATOM 2261 C VAL A 271 42.819 33.370 26.442 1.00 24.89 C
ATOM 2262 O VAL A 271 43.923 33.115 25.980 1.00 21.98 O
ATOM 2263 CB VAL A 271 42.901 35.813 26.736 1.00 29.25 C
ATOM 2264 CG1 VAL A 271 42.256 35.773 25.370 1.00 31.91 C
ATOM 2265 CG2 VAL A 271 42.421 36.989 27.565 1.00 18.72 C
ATOM 2266 N PHE A 272 41.716 32.758 26.019 1.00 26.14 N
ATOM 2267 CA PHE A 272 41.752 31.747 24.963 1.00 24.34 C
ATOM 2268 C PHE A 272 41.236 32.266 23.623 1.00 28.95 C
ATOM 2269 O PHE A 272 40.155 32.826 23.582 1.00 22.01 O
ATOM 2270 CB PHE A 272 40.960 30.506 25.391 1.00 20.97 C
ATOM 2271 CG PHE A 272 41.764 29.570 26.243 1.00 21.77 C
ATOM 2272 CD1 PHE A 272 41.940 29.842 27.610 1.00 14.60 C
ATOM 2273 CD2 PHE A 272 42.504 28.550 25.656 1.00 22.19 C
ATOM 2274 CE1 PHE A 272 42.763 29.041 28.434 1.00 17.89 C
ATOM 2275 CE2 PHE A 272 43.336 27.726 26.454 1.00 27.64 C
ATOM 2276 CZ PHE A 272 43.478 27.979 27.851 1.00 25.14 C
ATOM 2277 N ASP A 273 42.012 32.114 22.542 1.00 29.45 N
ATOM 2278 CA ASP A 273 41.557 32.536 21.214 1.00 22.33 C
ATOM 2279 C ASP A 273 40.896 31.365 20.493 1.00 25.67 C
ATOM 2280 O ASP A 273 41.539 30.570 19.793 1.00 17.81 O
ATOM 2281 CB ASP A 273 42.672 33.114 20.343 1.00 21.45 C
ATOM 2282 CG ASP A 273 42.131 33.626 18.990 1.00 26.89 C
ATOM 2283 OD1 ASP A 273 40.975 33.249 18.598 1.00 27.76 O
ATOM 2284 OD2 ASP A 273 42.838 34.421 18.327 1.00 30.06 O
ATOM 2285 N ALA A 274 39.589 31.284 20.649 1.00 15.59 N
ATOM 2286 CA ALA A 274 38.932 30.128 20.128 1.00 23.75 C
ATOM 2287 C ALA A 274 38.853 30.168 18.653 1.00 32.30 C
ATOM 2288 O ALA A 274 38.284 29.256 18.029 1.00 29.37 O
ATOM 2289 CB ALA A 274 37.567 29.905 20.777 1.00 18.87 C
ATOM 2290 N SER A 275 39.372 31.243 18.081 1.00 21.10 N
ATOM 2291 CA SER A 275 39.343 31.288 16.631 1.00 26.90 C
ATOM 2292 C SER A 275 40.390 30.300 16.116 1.00 43.37 C
ATOM 2293 O SER A 275 40.421 29.949 14.927 1.00 46.32 O
ATOM 2294 CB SER A 275 39.547 32.683 16.074 1.00 15.19 C
ATOM 2295 OG SER A 275 40.904 33.070 16.078 1.00 28.71 O
ATOM 2296 N LYS A 276 41.192 29.780 17.037 1.00 22.98 N
ATOM 2297 CA LYS A 276 42.178 28.791 16.638 1.00 23.28 C
ATOM 2298 C LYS A 276 41.645 27.405 16.976 1.00 29.73 C
ATOM 2299 O LYS A 276 40.992 27.206 18.010 1.00 25.10 O
ATOM 2300 CB LYS A 276 43.544 29.051 17.275 1.00 19.19 C
ATOM 2301 CG LYS A 276 43.957 30.496 17.218 1.00 32.11 C
ATOM 2302 CD LYS A 276 44.062 30.852 15.798 1.00 22.43 C
ATOM 2303 CE LYS A 276 44.930 32.067 15.570 1.00 23.18 C
ATOM 2304 NZ LYS A 276 45.454 32.117 14.152 1.00 29.42 N

ATOM 2305 N PRO A 277 41.892 26.476 16.055 1.00 36.04 N
ATOM 2306 CA PRO A 277 41.446 25.087 16.170 1.00 35.93 C
ATOM 2307 C PRO A 277 42.022 24.332 17.363 1.00 29.30 C
ATOM 2308 O PRO A 277 43.103 24.650 17.885 1.00 30.54 O
ATOM 2309 CB PRO A 277 41.975 24.453 14.878 1.00 39.65 C
ATOM 2310 CG PRO A 277 43.249 25.261 14.566 1.00 42.90 C
ATOM 2311 CD PRO A 277 42.787 26.670 14.892 1.00 37.84 C
ATOM 2312 N ASP A 278 41.273 23.339 17.809 1.00 22.35 N
ATOM 2313 CA ASP A 278 41.745 22.501 18.903 1.00 22.16 C
ATOM 2314 C ASP A 278 42.184 21.189 18.272 1.00 19.66 C
ATOM 2315 O ASP A 278 41.905 20.917 17.117 1.00 23.49 O
ATOM 2316 CB ASP A 278 40.636 22.241 19.971 1.00 15.09 C
ATOM 2317 CG ASP A 278 40.216 23.503 20.702 1.00 22.86 C
ATOM 2318 OD1 ASP A 278 41.113 24.254 21.096 1.00 25.18 O
ATOM 2319 OD2 ASP A 278 38.999 23.787 20.812 1.00 39.55 O
ATOM 2320 N GLY A 279 42.846 20.355 19.044 1.00 30.65 N
ATOM 2321 CA GLY A 279 43.229 19.034 18.546 1.00 33.78 C
ATOM 2322 C GLY A 279 42.115 18.099 18.944 1.00 38.10 C
ATOM 2323 O GLY A 279 40.963 18.517 19.068 1.00 47.52 O
ATOM 2324 N THR A 280 42.419 16.839 19.177 1.00 29.44 N
ATOM 2325 CA THR A 280 41.328 15.990 19.587 1.00 26.68 C
ATOM 2326 C THR A 280 40.889 16.439 20.972 1.00 23.52 C
ATOM 2327 O THR A 280 41.670 17.067 21.713 1.00 23.62 O
ATOM 2328 CB THR A 280 41.695 14.492 19.540 1.00 40.78 C
ATOM 2329 OG1 THR A 280 42.889 14.272 20.296 1.00 25.56 O
ATOM 2330 CG2 THR A 280 41.893 14.054 18.095 1.00 37.71 C
ATOM 2331 N PRO A 281 39.672 16.063 21.346 1.00 25.54 N
ATOM 2332 CA PRO A 281 39.129 16.454 22.628 1.00 25.72 C
ATOM 2333 C PRO A 281 39.776 15.778 23.800 1.00 26.02 C
ATOM 2334 O PRO A 281 39.752 16.314 24.915 1.00 22.68 O
ATOM 2335 CB PRO A 281 37.650 15.990 22.559 1.00 28.89 C
ATOM 2336 CG PRO A 281 37.417 15.540 21.201 1.00 29.39 C
ATOM 2337 CD PRO A 281 38.761 15.138 20.646 1.00 26.82 C
ATOM 2338 N ARG A 282 40.281 14.567 23.587 1.00 27.88 N
ATOM 2339 CA ARG A 282 40.806 13.817 24.720 1.00 34.08 C
ATOM 2340 C ARG A 282 41.977 12.918 24.384 1.00 27.62 C
ATOM 2341 O ARG A 282 41.913 12.182 23.425 1.00 23.83 O
ATOM 2342 CB ARG A 282 39.676 13.017 25.405 1.00 20.89 C
ATOM 2343 CG ARG A 282 40.035 12.467 26.775 1.00 22.81 C
ATOM 2344 CD ARG A 282 38.762 11.925 27.442 1.00 26.77 C
ATOM 2345 NE ARG A 282 38.963 11.345 28.781 1.00 36.48 N
ATOM 2346 CZ ARG A 282 38.518 10.139 29.164 1.00 37.74 C
ATOM 2347 NH1 ARG A 282 37.813 9.360 28.346 1.00 28.45 N
ATOM 2348 NH2 ARG A 282 38.754 9.700 30.384 1.00 27.25 N
ATOM 2349 N LYS A 283 43.016 12.963 25.223 1.00 28.91 N

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ATOM   2350  CA  LYS A 283      44.217  12.171  25.051  1.00 24.32           C
ATOM   2351  C   LYS A 283      44.796  11.766  26.404  1.00 29.57           C
ATOM   2352  O   LYS A 283      45.262  12.626  27.138  1.00 33.16           O
ATOM   2353  CB  LYS A 283      45.226  13.008  24.287  1.00 21.93           C
ATOM   2354  CG  LYS A 283      46.111  12.251  23.316  1.00 32.38           C
ATOM   2355  CD  LYS A 283      46.526  13.171  22.143  1.00 95.77           C
ATOM   2356  CE  LYS A 283      45.710  12.937  20.836  1.00100.00           C
ATOM   2357  NZ  LYS A 283      46.418  13.332  19.535  1.00100.00           N
ATOM   2358  N   LEU A 284      44.747  10.467  26.734  1.00 23.37           N
ATOM   2359  CA  LEU A 284      45.327   9.905  27.997  1.00 16.08           C
ATOM   2360  C   LEU A 284      45.463   8.386  28.047  1.00 20.46           C
ATOM   2361  O   LEU A 284      44.679   7.655  27.446  1.00 25.45           O
ATOM   2362  CB  LEU A 284      44.641  10.387  29.284  1.00 16.30           C
ATOM   2363  CG  LEU A 284      43.334   9.700  29.714  1.00 25.97           C
ATOM   2364  CD1 LEU A 284      42.881  10.089  31.152  1.00 22.11           C
ATOM   2365  CD2 LEU A 284      42.203   9.953  28.693  1.00 23.92           C
ATOM   2366  N   LEU A 285      46.453   7.939  28.820  1.00 18.51           N
ATOM   2367  CA  LEU A 285      46.792   6.527  29.003  1.00 16.77           C
ATOM   2368  C   LEU A 285      45.880   5.865  30.006  1.00 30.75           C
ATOM   2369  O   LEU A 285      45.576   6.439  31.058  1.00 22.02           O
ATOM   2370  CB  LEU A 285      48.229   6.389  29.585  1.00 15.85           C
ATOM   2371  CG  LEU A 285      49.307   6.970  28.672  1.00 21.51           C
ATOM   2372  CD1 LEU A 285      50.703   6.705  29.122  1.00 15.15           C
ATOM   2373  CD2 LEU A 285      49.051   6.368  27.330  1.00 16.94           C
ATOM   2374  N   ASP A 286      45.565   4.599  29.734  1.00 26.62           N
ATOM   2375  CA  ASP A 286      44.945   3.726  30.698  1.00 10.90           C
ATOM   2376  C   ASP A 286      46.128   3.055  31.498  1.00 20.54           C
ATOM   2377  O   ASP A 286      46.991   2.372  30.938  1.00 23.38           O
ATOM   2378  CB  ASP A 286      44.073   2.702  29.970  1.00 14.65           C
ATOM   2379  CG  ASP A 286      43.409   1.699  30.943  1.00 24.60           C
ATOM   2380  OD1 ASP A 286      43.932   1.437  32.083  1.00 24.60           O
ATOM   2381  OD2 ASP A 286      42.316   1.231  30.583  1.00 26.03           O
ATOM   2382  N   VAL A 287      46.230   3.317  32.791  1.00 15.44           N
ATOM   2383  CA  VAL A 287      47.354   2.816  33.556  1.00 15.58           C
ATOM   2384  C   VAL A 287      46.973   1.695  34.521  1.00 16.48           C
ATOM   2385  O   VAL A 287      47.613   1.473  35.572  1.00 16.63           O
ATOM   2386  CB  VAL A 287      48.101   4.006  34.260  1.00 29.84           C
ATOM   2387  CG1 VAL A 287      48.534   5.085  33.224  1.00 18.39           C
ATOM   2388  CG2 VAL A 287      47.173   4.670  35.258  1.00 37.79           C
ATOM   2389  N   THR A 288      45.904   0.992  34.152  1.00 22.27           N
ATOM   2390  CA  THR A 288      45.428  -0.152  34.956  1.00 19.34           C
ATOM   2391  C   THR A 288      46.561  -1.177  35.227  1.00 27.47           C
ATOM   2392  O   THR A 288      46.778  -1.586  36.365  1.00 24.87           O
ATOM   2393  CB  THR A 288      44.288  -0.909  34.244  1.00 22.86           C
ATOM   2394  OG1 THR A 288      43.120  -0.096  34.106  1.00 24.84           O
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ATOM	2395	CG2	THR	A 288	43.916	-2.113	35.024	1.00 25.08	C
ATOM	2396	N	ARG	A 289	47.290	-1.585	34.179	1.00 26.08	N
ATOM	2397	CA	ARG	A 289	48.428	-2.506	34.319	1.00 16.92	C
ATOM	2398	C	ARG	A 289	49.405	-2.037	35.408	1.00 22.96	C
ATOM	2399	O	ARG	A 289	49.847	-2.790	36.275	1.00 23.03	O
ATOM	2400	CB	ARG	A 289	49.208	-2.607	32.976	1.00 12.43	C
ATOM	2401	CG	ARG	A 289	48.934	-3.804	32.103	1.00 29.39	C
ATOM	2402	CD	ARG	A 289	50.016	-4.102	31.037	1.00 25.88	C
ATOM	2403	NE	ARG	A 289	49.441	-4.996	30.020	1.00 17.26	N
ATOM	2404	CZ	ARG	A 289	50.053	-5.459	28.930	1.00 38.82	C
ATOM	2405	NH1	ARG	A 289	51.306	-5.153	28.660	1.00 13.51	N
ATOM	2406	NH2	ARG	A 289	49.400	-6.262	28.096	1.00 37.68	N
ATOM	2407	N	LEU	A 290	49.815	-0.786	35.306	1.00 26.60	N
ATOM	2408	CA	LEU	A 290	50.809	-0.254	36.219	1.00 25.42	C
ATOM	2409	C	LEU	A 290	50.324	-0.376	37.656	1.00 24.17	C
ATOM	2410	O	LEU	A 290	51.072	-0.759	38.574	1.00 19.94	O
ATOM	2411	CB	LEU	A 290	51.000	1.219	35.876	1.00 24.66	C
ATOM	2412	CG	LEU	A 290	52.281	2.019	36.066	1.00 24.67	C
ATOM	2413	CD1	LEU	A 290	51.992	3.479	36.504	1.00 29.25	C
ATOM	2414	CD2	LEU	A 290	53.450	1.335	36.788	1.00 15.82	C
ATOM	2415	N	HIS	A 291	49.093	0.075	37.868	1.00 30.10	N
ATOM	2416	CA	HIS	A 291	48.513	0.074	39.212	1.00 34.17	C
ATOM	2417	C	HIS	A 291	48.411	-1.367	39.730	1.00 43.41	C
ATOM	2418	O	HIS	A 291	48.621	-1.654	40.929	1.00 38.81	O
ATOM	2419	CB	HIS	A 291	47.113	0.674	39.143	1.00 28.01	C
ATOM	2420	CG	HIS	A 291	47.097	2.153	38.984	1.00 29.68	C
ATOM	2421	ND1	HIS	A 291	48.242	2.921	39.015	1.00 35.63	N
ATOM	2422	CD2	HIS	A 291	46.068	3.024	38.855	1.00 31.18	C
ATOM	2423	CE1	HIS	A 291	47.926	4.197	38.845	1.00 24.20	C
ATOM	2424	NE2	HIS	A 291	46.612	4.289	38.747	1.00 21.92	N
ATOM	2425	N	GLN	A 292	48.048	-2.260	38.821	1.00 30.71	N
ATOM	2426	CA	GLN	A 292	47.950	-3.654	39.181	1.00 34.82	C
ATOM	2427	C	GLN	A 292	49.287	-4.197	39.622	1.00 36.93	C
ATOM	2428	O	GLN	A 292	49.323	-5.040	40.510	1.00 27.56	O
ATOM	2429	CB	GLN	A 292	47.322	-4.487	38.069	1.00 28.23	C
ATOM	2430	CG	GLN	A 292	45.798	-4.405	38.171	1.00 81.15	C
ATOM	2431	CD	GLN	A 292	45.023	-4.954	36.963	1.00100.00	C
ATOM	2432	OE1	GLN	A 292	45.597	-5.410	35.951	1.00 99.65	O
ATOM	2433	NE2	GLN	A 292	43.687	-4.895	37.073	1.00 40.86	N
ATOM	2434	N	LEU	A 293	50.375	-3.658	39.058	1.00 31.75	N
ATOM	2435	CA	LEU	A 293	51.750	-4.072	39.383	1.00 22.67	C
ATOM	2436	C	LEU	A 293	52.238	-3.323	40.613	1.00 28.64	C
ATOM	2437	O	LEU	A 293	53.420	-3.377	41.017	1.00 22.27	O
ATOM	2438	CB	LEU	A 293	52.665	-3.769	38.205	1.00 25.57	C
ATOM	2439	CG	LEU	A 293	52.497	-4.703	37.016	1.00 35.11	C

ATOM	2440	CD1	LEU A 293	53.306	-4.170	35.836	1.00	28.25	C
ATOM	2441	CD2	LEU A 293	52.965	-6.110	37.439	1.00	47.81	C
ATOM	2442	N	GLY A 294	51.316	-2.510	41.111	1.00	33.08	N
ATOM	2443	CA	GLY A 294	51.488	-1.793	42.347	1.00	24.90	C
ATOM	2444	C	GLY A 294	52.272	-0.512	42.326	1.00	29.31	C
ATOM	2445	O	GLY A 294	53.070	-0.249	43.223	1.00	25.25	O
ATOM	2446	N	TRP A 295	52.000	0.347	41.368	1.00	27.83	N
ATOM	2447	CA	TRP A 295	52.687	1.623	41.385	1.00	19.45	C
ATOM	2448	C	TRP A 295	51.684	2.731	41.081	1.00	25.79	C
ATOM	2449	O	TRP A 295	50.765	2.527	40.297	1.00	20.43	O
ATOM	2450	CB	TRP A 295	53.961	1.614	40.524	1.00	12.85	C
ATOM	2451	CG	TRP A 295	54.750	2.911	40.618	1.00	23.04	C
ATOM	2452	CD1	TRP A 295	55.897	3.161	41.368	1.00	23.68	C
ATOM	2453	CD2	TRP A 295	54.415	4.159	39.979	1.00	20.72	C
ATOM	2454	NE1	TRP A 295	56.258	4.493	41.244	1.00	18.67	N
ATOM	2455	CE2	TRP A 295	55.389	5.113	40.373	1.00	20.95	C
ATOM	2456	CE3	TRP A 295	53.406	4.550	39.102	1.00	21.47	C
ATOM	2457	CZ2	TRP A 295	55.338	6.439	39.958	1.00	17.58	C
ATOM	2458	CZ3	TRP A 295	53.403	5.873	38.632	1.00	21.57	C
ATOM	2459	CH2	TRP A 295	54.368	6.787	39.058	1.00	19.45	C
ATOM	2460	N	TYR A 296	51.709	3.797	41.884	1.00	25.17	N
ATOM	2461	CA	TYR A 296	50.720	4.883	41.731	1.00	24.90	C
ATOM	2462	C	TYR A 296	51.517	6.178	41.857	1.00	30.85	C
ATOM	2463	O	TYR A 296	52.363	6.272	42.745	1.00	21.27	O
ATOM	2464	CB	TYR A 296	49.654	4.813	42.840	1.00	25.18	C
ATOM	2465	CG	TYR A 296	48.685	3.651	42.744	1.00	23.04	C
ATOM	2466	CD1	TYR A 296	49.078	2.343	43.088	1.00	31.62	C
ATOM	2467	CD2	TYR A 296	47.380	3.853	42.289	1.00	26.02	C
ATOM	2468	CE1	TYR A 296	48.203	1.268	42.935	1.00	24.42	C
ATOM	2469	CE2	TYR A 296	46.493	2.770	42.127	1.00	24.81	C
ATOM	2470	CZ	TYR A 296	46.902	1.483	42.464	1.00	39.41	C
ATOM	2471	OH	TYR A 296	45.984	0.434	42.337	1.00	66.19	O
ATOM	2472	N	HIS A 297	51.324	7.123	40.924	1.00	20.95	N
ATOM	2473	CA	HIS A 297	52.130	8.343	40.938	1.00	26.86	C
ATOM	2474	C	HIS A 297	51.947	9.175	42.210	1.00	35.01	C
ATOM	2475	O	HIS A 297	50.885	9.132	42.874	1.00	26.92	O
ATOM	2476	CB	HIS A 297	51.819	9.192	39.733	1.00	25.77	C
ATOM	2477	CG	HIS A 297	50.489	9.842	39.803	1.00	31.16	C
ATOM	2478	ND1	HIS A 297	49.314	9.145	39.633	1.00	34.21	N
ATOM	2479	CD2	HIS A 297	50.135	11.094	40.167	1.00	25.83	C
ATOM	2480	CE1	HIS A 297	48.290	9.972	39.776	1.00	24.14	C
ATOM	2481	NE2	HIS A 297	48.761	11.164	40.087	1.00	23.35	N
ATOM	2482	N	GLU A 298	52.983	9.926	42.554	1.00	24.98	N
ATOM	2483	CA	GLU A 298	52.957	10.683	43.798	1.00	27.65	C
ATOM	2484	C	GLU A 298	52.831	12.187	43.741	1.00	36.86	C

ATOM 2485 O GLU A 298 52.433 12.792 44.718 1.00 43.61 O
ATOM 2486 CB GLU A 298 54.153 10.319 44.686 1.00 22.02 C
ATOM 2487 CG GLU A 298 54.004 8.943 45.285 1.00 36.42 C
ATOM 2488 CD GLU A 298 54.999 8.664 46.406 1.00100.00 C
ATOM 2489 OE1 GLU A 298 56.223 8.561 46.152 1.00 44.79 O
ATOM 2490 OE2 GLU A 298 54.526 8.470 47.547 1.00100.00 O
ATOM 2491 N ILE A 299 53.232 12.800 42.639 1.00 23.49 N
ATOM 2492 CA ILE A 299 53.268 14.244 42.562 1.00 13.25 C
ATOM 2493 C ILE A 299 52.016 14.848 41.906 1.00 27.05 C
ATOM 2494 O ILE A 299 51.681 14.530 40.757 1.00 26.73 O
ATOM 2495 CB ILE A 299 54.586 14.711 41.862 1.00 15.93 C
ATOM 2496 CG1 ILE A 299 55.836 14.183 42.606 1.00 23.83 C
ATOM 2497 CG2 ILE A 299 54.596 16.213 41.541 1.00 17.37 C
ATOM 2498 CD1 ILE A 299 57.232 14.221 41.787 1.00 21.32 C
ATOM 2499 N SER A 300 51.323 15.716 42.648 1.00 18.55 N
ATOM 2500 CA SER A 300 50.177 16.449 42.091 1.00 19.58 C
ATOM 2501 C SER A 300 50.714 17.415 41.042 1.00 17.29 C
ATOM 2502 O SER A 300 51.824 17.941 41.178 1.00 21.06 O
ATOM 2503 CB SER A 300 49.542 17.307 43.181 1.00 16.78 C
ATOM 2504 OG SER A 300 50.548 17.969 43.923 1.00 75.80 O
ATOM 2505 N LEU A 301 49.870 17.755 40.075 1.00 16.13 N
ATOM 2506 CA LEU A 301 50.246 18.675 39.014 1.00 17.70 C
ATOM 2507 C LEU A 301 50.689 19.964 39.646 1.00 20.11 C
ATOM 2508 O LEU A 301 51.714 20.568 39.303 1.00 20.46 O
ATOM 2509 CB LEU A 301 48.990 18.981 38.197 1.00 17.92 C
ATOM 2510 CG LEU A 301 49.182 20.030 37.112 1.00 25.15 C
ATOM 2511 CD1 LEU A 301 50.233 19.552 36.086 1.00 18.82 C
ATOM 2512 CD2 LEU A 301 47.854 20.177 36.436 1.00 25.88 C
ATOM 2513 N GLU A 302 49.845 20.398 40.554 1.00 27.01 N
ATOM 2514 CA GLU A 302 50.053 21.636 41.280 1.00 37.72 C
ATOM 2515 C GLU A 302 51.410 21.618 41.996 1.00 29.99 C
ATOM 2516 O GLU A 302 52.245 22.514 41.798 1.00 27.15 O
ATOM 2517 CB GLU A 302 48.899 21.841 42.275 1.00 43.10 C
ATOM 2518 CG GLU A 302 49.061 23.061 43.174 1.00 90.85 C
ATOM 2519 CD GLU A 302 48.451 24.324 42.580 1.00100.00 C
ATOM 2520 OE1 GLU A 302 47.566 24.209 41.706 1.00100.00 O
ATOM 2521 OE2 GLU A 302 48.808 25.432 43.036 1.00 64.50 O
ATOM 2522 N ALA A 303 51.646 20.591 42.801 1.00 8.72 N
ATOM 2523 CA ALA A 303 52.937 20.455 43.459 1.00 15.03 C
ATOM 2524 C ALA A 303 54.102 20.355 42.450 1.00 19.85 C
ATOM 2525 O ALA A 303 55.104 21.090 42.553 1.00 22.24 O
ATOM 2526 CB ALA A 303 52.938 19.258 44.410 1.00 18.97 C
ATOM 2527 N GLY A 304 53.953 19.472 41.467 1.00 13.05 N
ATOM 2528 CA GLY A 304 54.970 19.321 40.448 1.00 8.94 C
ATOM 2529 C GLY A 304 55.239 20.621 39.695 1.00 20.31 C

ATOM	2530	O	GLY A 304	56.394	20.900	39.322	1.00	14.30	O
ATOM	2531	N	LEU A 305	54.191	21.383	39.361	1.00	10.76	N
ATOM	2532	CA	LEU A 305	54.483	22.622	38.611	1.00	20.29	C
ATOM	2533	C	LEU A 305	55.281	23.669	39.456	1.00	28.92	C
ATOM	2534	O	LEU A 305	56.194	24.385	38.974	1.00	17.69	O
ATOM	2535	CB	LEU A 305	53.202	23.245	38.033	1.00	24.03	C
ATOM	2536	CG	LEU A 305	52.357	22.647	36.880	1.00	27.66	C
ATOM	2537	CD1	LEU A 305	50.975	23.384	36.789	1.00	13.44	C
ATOM	2538	CD2	LEU A 305	53.079	22.724	35.543	1.00	18.39	C
ATOM	2539	N	ALA A 306	54.904	23.757	40.724	1.00	19.94	N
ATOM	2540	CA	ALA A 306	55.544	24.660	41.655	1.00	24.79	C
ATOM	2541	C	ALA A 306	57.035	24.380	41.743	1.00	27.51	C
ATOM	2542	O	ALA A 306	57.852	25.280	41.662	1.00	29.68	O
ATOM	2543	CB	ALA A 306	54.937	24.471	43.002	1.00	17.87	C
ATOM	2544	N	SER A 307	57.378	23.137	42.011	1.00	18.46	N
ATOM	2545	CA	SER A 307	58.793	22.756	42.162	1.00	16.31	C
ATOM	2546	C	SER A 307	59.547	22.885	40.832	1.00	22.66	C
ATOM	2547	O	SER A 307	60.742	23.212	40.786	1.00	28.47	O
ATOM	2548	CB	SER A 307	58.851	21.304	42.622	1.00	20.47	C
ATOM	2549	OG	SER A 307	58.517	20.454	41.526	1.00	29.03	O
ATOM	2550	N	THR A 308	58.849	22.631	39.735	1.00	27.31	N
ATOM	2551	CA	THR A 308	59.458	22.738	38.413	1.00	22.89	C
ATOM	2552	C	THR A 308	59.757	24.216	38.107	1.00	26.06	C
ATOM	2553	O	THR A 308	60.819	24.546	37.591	1.00	29.89	O
ATOM	2554	CB	THR A 308	58.536	22.115	37.318	1.00	18.72	C
ATOM	2555	OG1	THR A 308	58.356	20.714	37.545	1.00	20.17	O
ATOM	2556	CG2	THR A 308	59.094	22.330	35.923	1.00	12.37	C
ATOM	2557	N	TYR A 309	58.846	25.118	38.453	1.00	28.20	N
ATOM	2558	CA	TYR A 309	59.110	26.549	38.241	1.00	31.09	C
ATOM	2559	C	TYR A 309	60.383	27.059	39.045	1.00	16.31	C
ATOM	2560	O	TYR A 309	61.179	27.858	38.577	1.00	16.91	O
ATOM	2561	CB	TYR A 309	57.819	27.373	38.533	1.00	31.19	C
ATOM	2562	CG	TYR A 309	57.944	28.895	38.392	1.00	14.57	C
ATOM	2563	CD1	TYR A 309	58.397	29.457	37.224	1.00	17.51	C
ATOM	2564	CD2	TYR A 309	57.575	29.757	39.442	1.00	24.99	C
ATOM	2565	CE1	TYR A 309	58.527	30.801	37.100	1.00	18.41	C
ATOM	2566	CE2	TYR A 309	57.744	31.129	39.351	1.00	19.04	C
ATOM	2567	CZ	TYR A 309	58.212	31.641	38.164	1.00	29.13	C
ATOM	2568	OH	TYR A 309	58.300	33.004	37.966	1.00	28.22	O
ATOM	2569	N	GLN A 310	60.560	26.579	40.260	1.00	15.41	N
ATOM	2570	CA	GLN A 310	61.705	26.964	41.087	1.00	22.35	C
ATOM	2571	C	GLN A 310	63.001	26.492	40.446	1.00	31.46	C
ATOM	2572	O	GLN A 310	64.009	27.191	40.442	1.00	33.42	O
ATOM	2573	CB	GLN A 310	61.587	26.335	42.482	1.00	17.67	C
ATOM	2574	CG	GLN A 310	62.579	26.921	43.461	1.00	57.58	C

ATOM 2575 CD GLN A 310 62.287 28.370 43.782 1.00 65.14 C
ATOM 2576 OE1 GLN A 310 61.134 28.754 44.000 1.00 41.94 O
ATOM 2577 NE2 GLN A 310 63.330 29.194 43.801 1.00 99.09 N
ATOM 2578 N TRP A 311 62.957 25.321 39.830 1.00 28.76 N
ATOM 2579 CA TRP A 311 64.146 24.822 39.163 1.00 26.29 C
ATOM 2580 C TRP A 311 64.474 25.769 38.040 1.00 17.91 C
ATOM 2581 O TRP A 311 65.599 26.193 37.880 1.00 22.89 O
ATOM 2582 CB TRP A 311 63.938 23.383 38.643 1.00 27.53 C
ATOM 2583 CG TRP A 311 65.176 22.784 38.119 1.00 17.82 C
ATOM 2584 CD1 TRP A 311 66.132 22.090 38.826 1.00 20.21 C
ATOM 2585 CD2 TRP A 311 65.652 22.881 36.784 1.00 17.99 C
ATOM 2586 NE1 TRP A 311 67.197 21.776 37.992 1.00 20.39 N
ATOM 2587 CE2 TRP A 311 66.933 22.284 36.746 1.00 19.57 C
ATOM 2588 CE3 TRP A 311 65.141 23.461 35.621 1.00 20.26 C
ATOM 2589 CZ2 TRP A 311 67.686 22.236 35.599 1.00 14.25 C
ATOM 2590 CZ3 TRP A 311 65.901 23.446 34.501 1.00 18.59 C
ATOM 2591 CH2 TRP A 311 67.169 22.831 34.494 1.00 16.86 C
ATOM 2592 N PHE A 312 63.469 26.109 37.256 1.00 17.47 N
ATOM 2593 CA PHE A 312 63.665 27.064 36.179 1.00 20.14 C
ATOM 2594 C PHE A 312 64.224 28.371 36.733 1.00 18.33 C
ATOM 2595 O PHE A 312 65.080 29.024 36.104 1.00 24.76 O
ATOM 2596 CB PHE A 312 62.328 27.318 35.458 1.00 29.51 C
ATOM 2597 CG PHE A 312 62.328 28.544 34.603 1.00 28.52 C
ATOM 2598 CD1 PHE A 312 62.883 28.508 33.338 1.00 30.53 C
ATOM 2599 CD2 PHE A 312 61.825 29.758 35.104 1.00 29.31 C
ATOM 2600 CE1 PHE A 312 62.936 29.660 32.554 1.00 34.73 C
ATOM 2601 CE2 PHE A 312 61.900 30.904 34.362 1.00 38.40 C
ATOM 2602 CZ PHE A 312 62.432 30.860 33.063 1.00 40.73 C
ATOM 2603 N LEU A 313 63.697 28.787 37.876 1.00 22.46 N
ATOM 2604 CA LEU A 313 64.170 30.025 38.516 1.00 28.47 C
ATOM 2605 C LEU A 313 65.627 29.827 38.898 1.00 37.53 C
ATOM 2606 O LEU A 313 66.452 30.693 38.629 1.00 34.20 O
ATOM 2607 CB LEU A 313 63.375 30.410 39.783 1.00 20.44 C
ATOM 2608 CG LEU A 313 61.955 30.897 39.555 1.00 16.29 C
ATOM 2609 CD1 LEU A 313 61.499 31.399 40.871 1.00 15.94 C
ATOM 2610 CD2 LEU A 313 61.959 31.961 38.524 1.00 14.44 C
ATOM 2611 N GLU A 314 65.953 28.685 39.508 1.00 30.70 N
ATOM 2612 CA GLU A 314 67.353 28.432 39.875 1.00 24.15 C
ATOM 2613 C GLU A 314 68.291 28.149 38.703 1.00 36.34 C
ATOM 2614 O GLU A 314 69.485 28.047 38.890 1.00 43.10 O
ATOM 2615 CB GLU A 314 67.459 27.366 40.947 1.00 19.90 C
ATOM 2616 CG GLU A 314 66.634 27.754 42.141 1.00 27.37 C
ATOM 2617 CD GLU A 314 66.450 26.666 43.182 1.00 31.09 C
ATOM 2618 OE1 GLU A 314 67.157 25.648 43.085 1.00 59.60 O
ATOM 2619 OE2 GLU A 314 65.634 26.872 44.125 1.00 46.20 O

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ATOM   2620  N   ASN A 315      67.778  28.114  37.479  1.00 40.17           N
ATOM   2621  CA  ASN A 315      68.637  27.802  36.343  1.00 37.76           C
ATOM   2622  C   ASN A 315      68.383  28.578  35.112  1.00 43.75           C
ATOM   2623  O   ASN A 315      68.591  28.001  34.047  1.00 39.15           O
ATOM   2624  CB  ASN A 315      68.425  26.360  35.884  1.00 33.74           C
ATOM   2625  CG  ASN A 315      69.028  25.383  36.801  1.00 53.18           C
ATOM   2626  OD1 ASN A 315      68.456  25.087  37.835  1.00 49.13           O
ATOM   2627  ND2 ASN A 315      70.239  24.926  36.479  1.00 97.72           N
ATOM   2628  N   GLN A 316      67.852  29.803  35.197  1.00 49.87           N
ATOM   2629  CA  GLN A 316      67.627  30.550  33.957  1.00 77.90           C
ATOM   2630  C   GLN A 316      68.797  31.448  33.525  1.00100.00           C
ATOM   2631  O   GLN A 316      69.272  31.387  32.375  1.00 51.33           O
ATOM   2632  CB  GLN A 316      66.280  31.276  33.902  1.00 75.89           C
ATOM   2633  CG  GLN A 316      65.683  31.589  35.231  1.00 80.97           C
ATOM   2634  CD  GLN A 316      65.233  33.036  35.350  1.00 54.58           C
ATOM   2635  OE1 GLN A 316      64.881  33.699  34.367  1.00 46.46           O
ATOM   2636  NE2 GLN A 316      65.257  33.538  36.566  1.00 33.46           N
TER    2637       GLN A 316
CONECT  110  111
CONECT  111  110  112
CONECT  112  111  113  114
CONECT  113  112  118
CONECT  114  112  115  116
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CONECT  129  117  130  131  132
CONECT  130  129
CONECT  131  129
CONECT  132  129
MASTER      208    0    1   13   10    0    3    6 2636    1   22   25
END
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While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. However, it is to be expressly understood that such modifications and adaptations are within the spirit and scope of the present invention, as set forth in the following claims.

What is claimed:

1. A method for producing ascorbic acid or esters thereof in a microorganism, comprising culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase; and recovering said ascorbic acid or esters thereof.

2. A method, as claimed in Claim 1, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

3. A method, as claimed in Claim 1, wherein said genetic modification is a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose.

4. A method, as claimed in Claim 3, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

5. The method of Claim 3, wherein said genetic modification comprises transformation of said microorganism with a recombinant nucleic acid molecule that expresses said epimerase.

6. The method of Claim 5, wherein said epimerase has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

7. The method of Claim 5, wherein said epimerase has a structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

8. The method of Claim 5, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 1 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

9. The method of Claim 5, wherein said epimerase comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

10. The method of Claim 9, wherein said substrate binding site has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

11. The method of Claim 5, wherein said epimerase comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

12. The method of Claim 11, wherein said catalytic site has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

13. The method of Claim 11, wherein said catalytic site comprises the amino acid residues serine, tyrosine and lysine.

14. The method of Claim 13, wherein tertiary structure positions of said amino acid residues serine, tyrosine and lysine substantially conform to tertiary structure positions of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws.

15. The method of Claim 5, wherein said epimerase binds NADPH.

16. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

17. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 75% of non-Xaa residues in SEQ ID NO:11.

18. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 90% of non-Xaa residues in SEQ ID NO:11.

19. The method of Claim 5, wherein said epimerase comprises an amino acid sequence having at least 4 contiguous amino acid residues that are 100% identical to at least 4 contiguous amino acid residues of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.

20. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence comprising at least about 12 contiguous nucleotides having 100% identity with at least about 12 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

21. The method of Claim 5, wherein said epimerase comprises an amino acid sequence having a motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly.

22. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 15% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

23. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 20% identical to a nucleic acid

sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

24. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 25% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

25. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase.

26. The method of Claim 25, wherein said nucleic acid sequence encoding said GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5.

27. The method of Claim 25, wherein said GDP-4-keto-6-deoxy-D-mannose epimerase/reductase comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

28. A method, as claimed in Claim 1, wherein said microorganism is selected from the group consisting of bacteria, fungi and microalgae.

29. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant.

30. A method, as claimed in Claim 1, wherein said microorganism is a bacterium.

31. A method, as claimed in Claim 30, wherein said bacterium is selected from the group consisting of *Azotobacter* and *Pseudomonas*.

32. A method, as claimed in Claim 1, wherein said microorganism is a fungus.

33. A method, as claimed in Claim 32, wherein said microorganism is a yeast.

34. A method, as claimed in Claim 33, wherein said yeast is selected from the group consisting of *Saccharomyces* yeast.

35. A method, as claimed in Claim 1, wherein said microorganism is a microalga.

36. A method, as claimed in Claim 35, wherein said microalga is selected from the group consisting of microalgae of the genera *Prototheca* and *Chlorella*.

37. A method, as claimed in Claim 36, wherein said microalga is selected from the genus *Prototheca*.

38. A method, as claimed in Claim 1, wherein said microorganism further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase.

39. A method, as claimed in Claim 38, wherein said genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase is a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

40. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 6.0.

41. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 5.5.

42. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 5.0.

43. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that is magnesium (Mg) limited.

44. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that is Mg limited during a cell growth phase.

45. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.5 g/L of Mg during a cell growth phase.

46. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.2 g/L of Mg during a cell growth phase.

47. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.1 g/L of Mg during a cell growth phase.

48. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises a carbon source other than D-mannose.

49. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises glucose as a carbon source.

50. A microorganism for producing ascorbic acid or esters thereof, wherein said microorganism has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

51. A microorganism, as claimed in Claim 50, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

52. A microorganism, as claimed in Claim 50, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

53. A microorganism, as claimed in Claim 50, wherein said microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

54. A microorganism, as claimed in Claim 50, wherein said microorganism is selected from the group consisting of bacteria, fungi and microalgae.

55. A microorganism, as claimed in Claim 50, wherein said microorganism is a bacterium.

56. A microorganism, as claimed in Claim 55, wherein said bacterium is selected from the group consisting of *Azotobacter* and *Pseudomonas*.

57. A microorganism, as claimed in Claim 50, wherein said microorganism is a fungus.

58. A microorganism, as claimed in Claim 57, wherein said microorganism is a yeast.

59. A microorganism, as claimed in Claim 58, wherein said yeast is selected from the group consisting of *Saccharomyces* yeast.

60. A plant for producing ascorbic acid or esters thereof, wherein said plant has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

61. A plant, as claimed in Claim 60, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

62. A plant, as claimed in Claim 60, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

63. A plant, as claimed in Claim 60, wherein said plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-

deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

64. A plant, as claimed in Claim 60, wherein said plant further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase.

65. A plant, as claimed in Claim 60, wherein said genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase is a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

66. A plant, as claimed in Claim 60, wherein said plant is a microalga.

67. A plant, as claimed in Claim 66, wherein said plant is selected from the group consisting of microalgae of the genera *Prototheca* and *Chlorella*.

68. A plant, as claimed in Claim 66, wherein said microalga is selected from the genus *Prototheca*.

69. A plant, as claimed in Claim 60, wherein said plant is a higher plant.

70. A plant, as claimed in Claim 60, wherein said plant is a consumable higher plant.

71. A microorganism for producing ascorbic acid or esters thereof, wherein said microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

72. A plant for producing ascorbic acid or esters thereof, wherein said plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

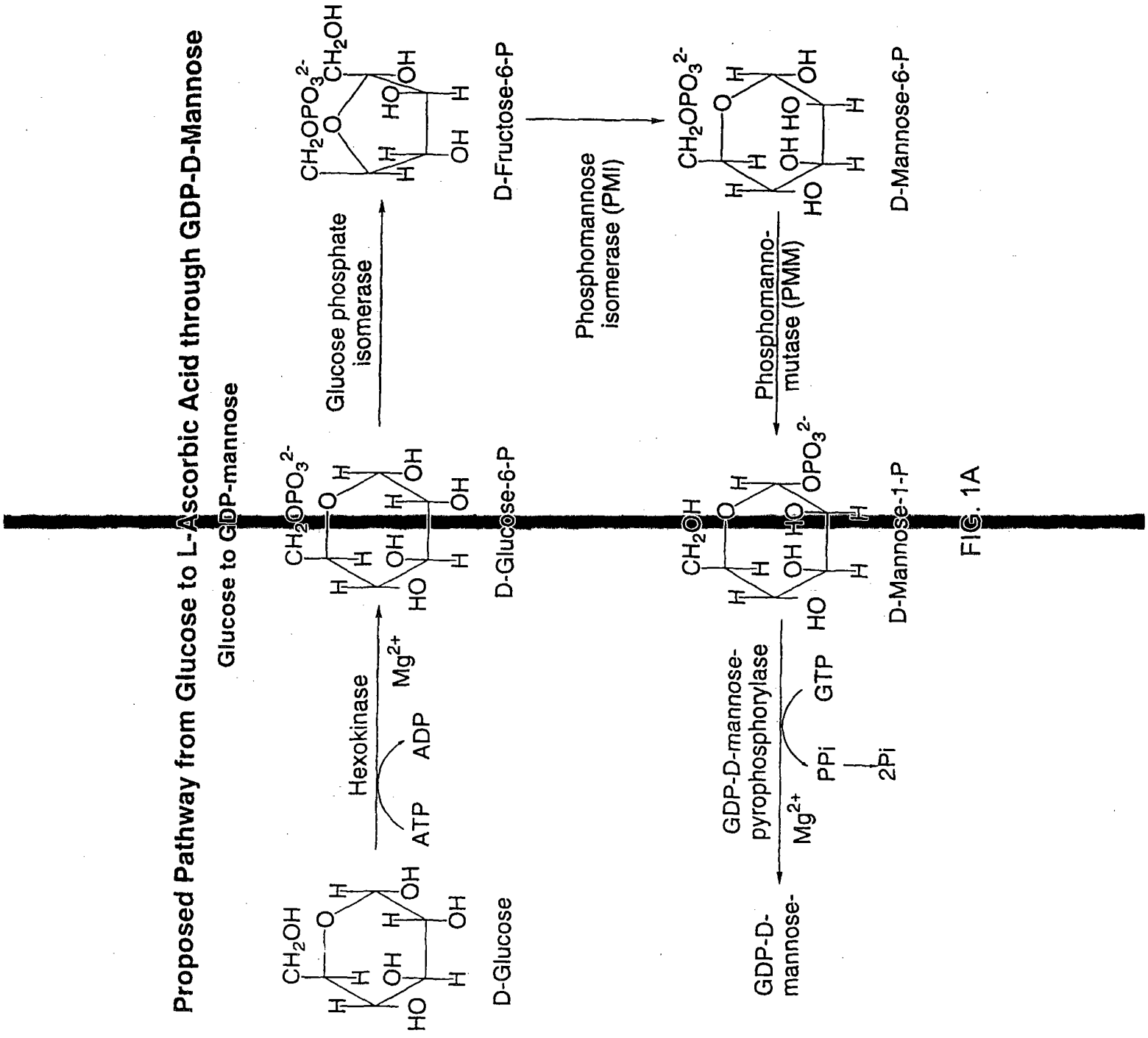
Proposed Pathway from Glucose to L-Ascorbic Acid through GDP-D-Mannose
Glucose to GDP-mannose
D-Glucose
Hexokinase
ATP
ADP
Mg^{2+}
D-Glucose-6-P
Glucose phosphate isomerase
D-Fructose-6-P
Phosphomannose isomerase (PMI)
D-Mannose-6-P
Phosphomanno-mutase (PMM)
D-Mannose-1-P
GDP-D-mannose-pyrophosphorylase
GTP
PPi
2Pi
Mg^{2+}
GDP-D-mannose-

FIG. 1A

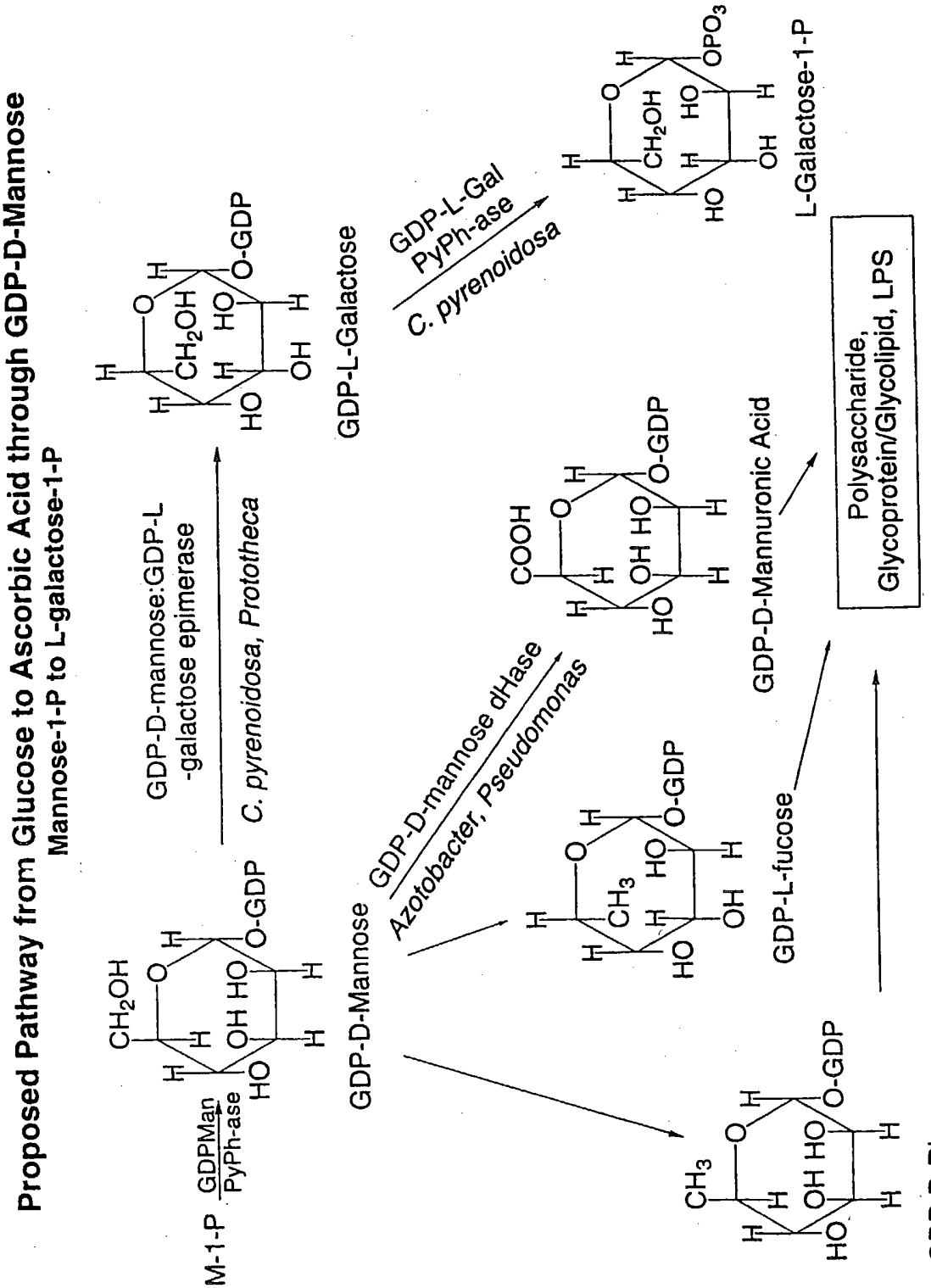

FIG. 1B

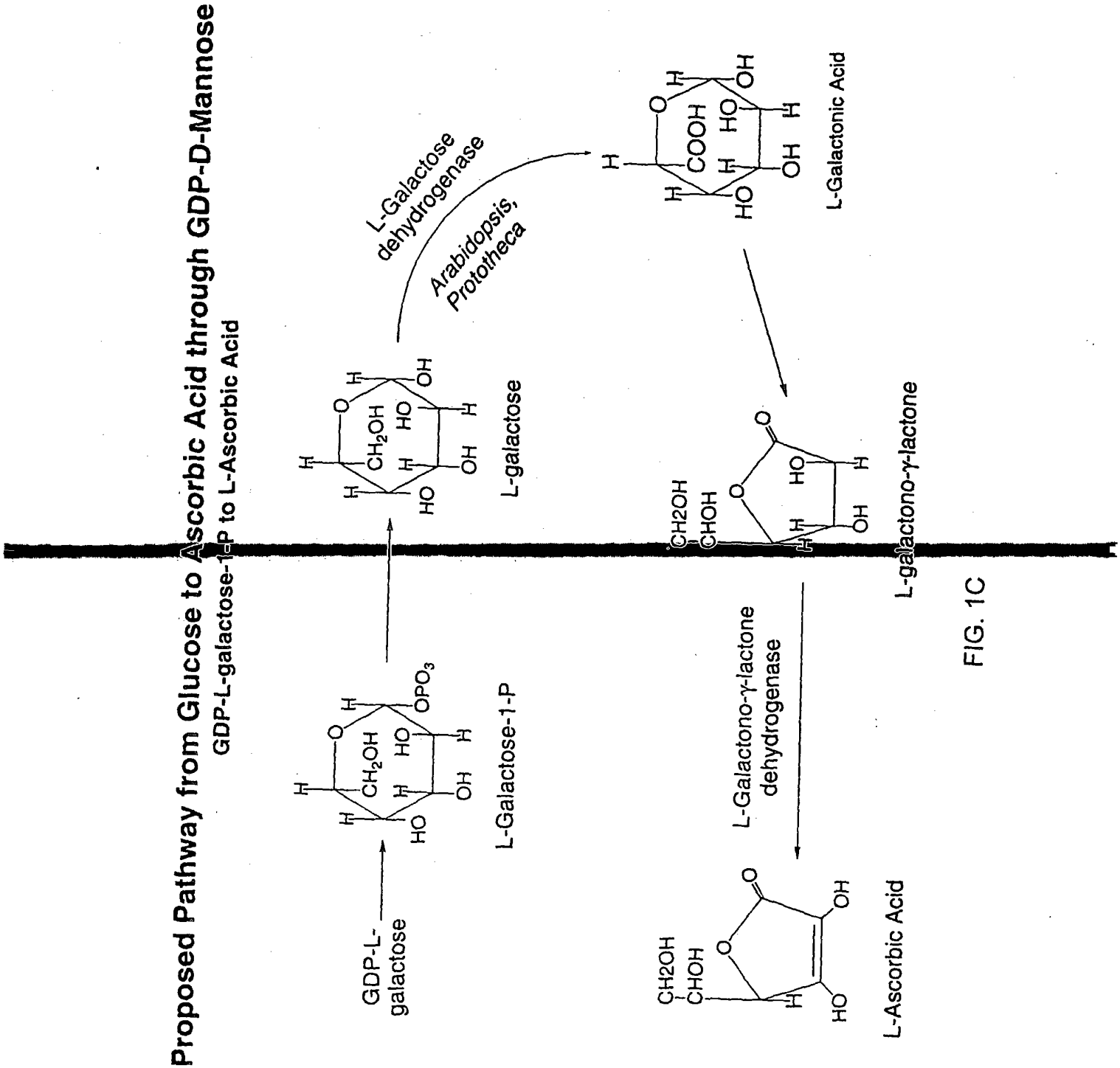
Proposed Pathway from Glucose to Ascorbic Acid through GDP-D-Mannose
GDP-L-galactose-1-P to L-Ascorbic Acid
GDP-L-galactose
L-Galactose-1-P
L-galactose
L-Galactose dehydrogenase
Arabidopsis, Prototheca
L-Galactonic Acid
L-galactono-γ-lactone
L-Galactono-γ-lactone dehydrogenase
L-Ascorbic Acid

FIG. 1C

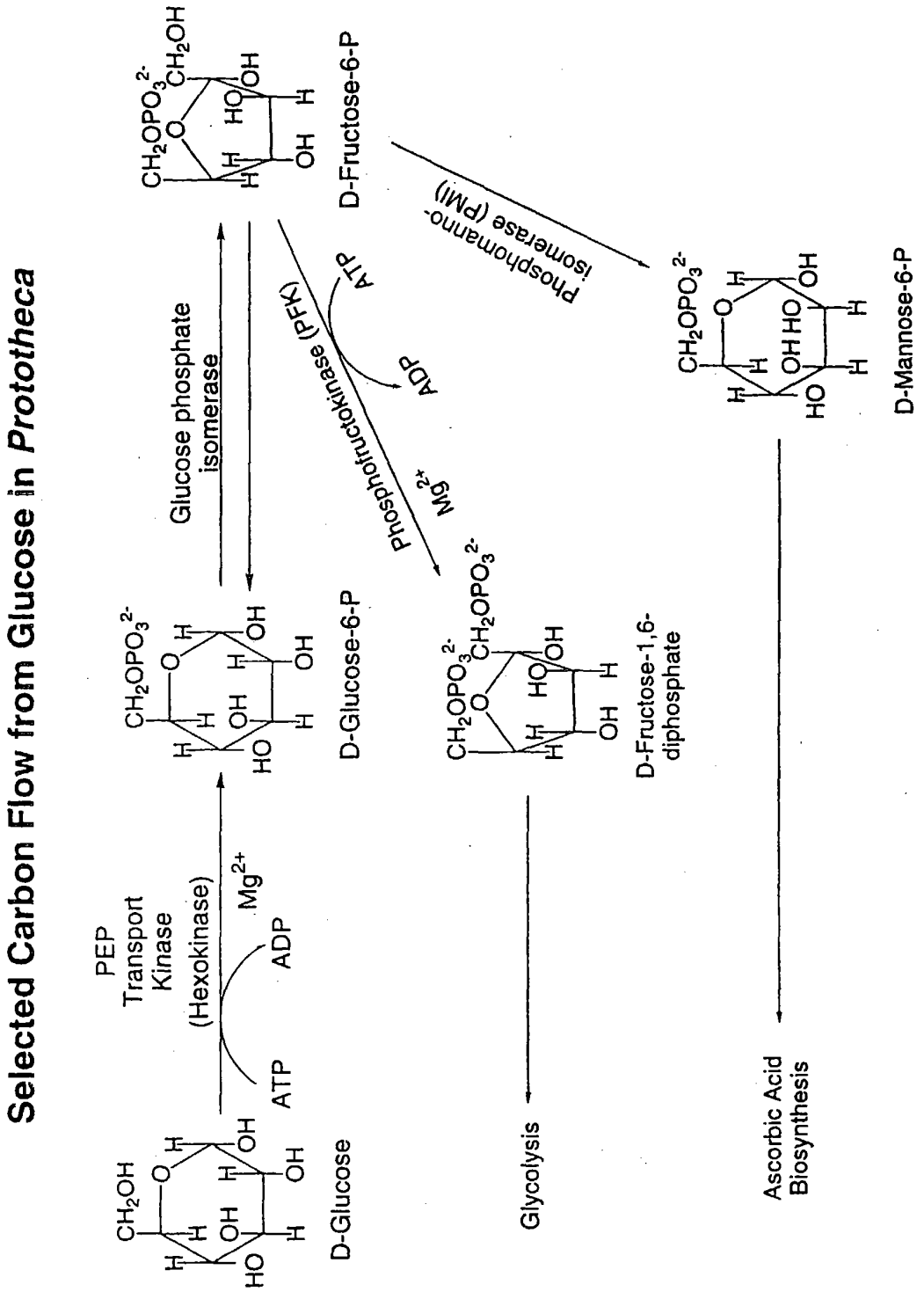

FIG. 2A

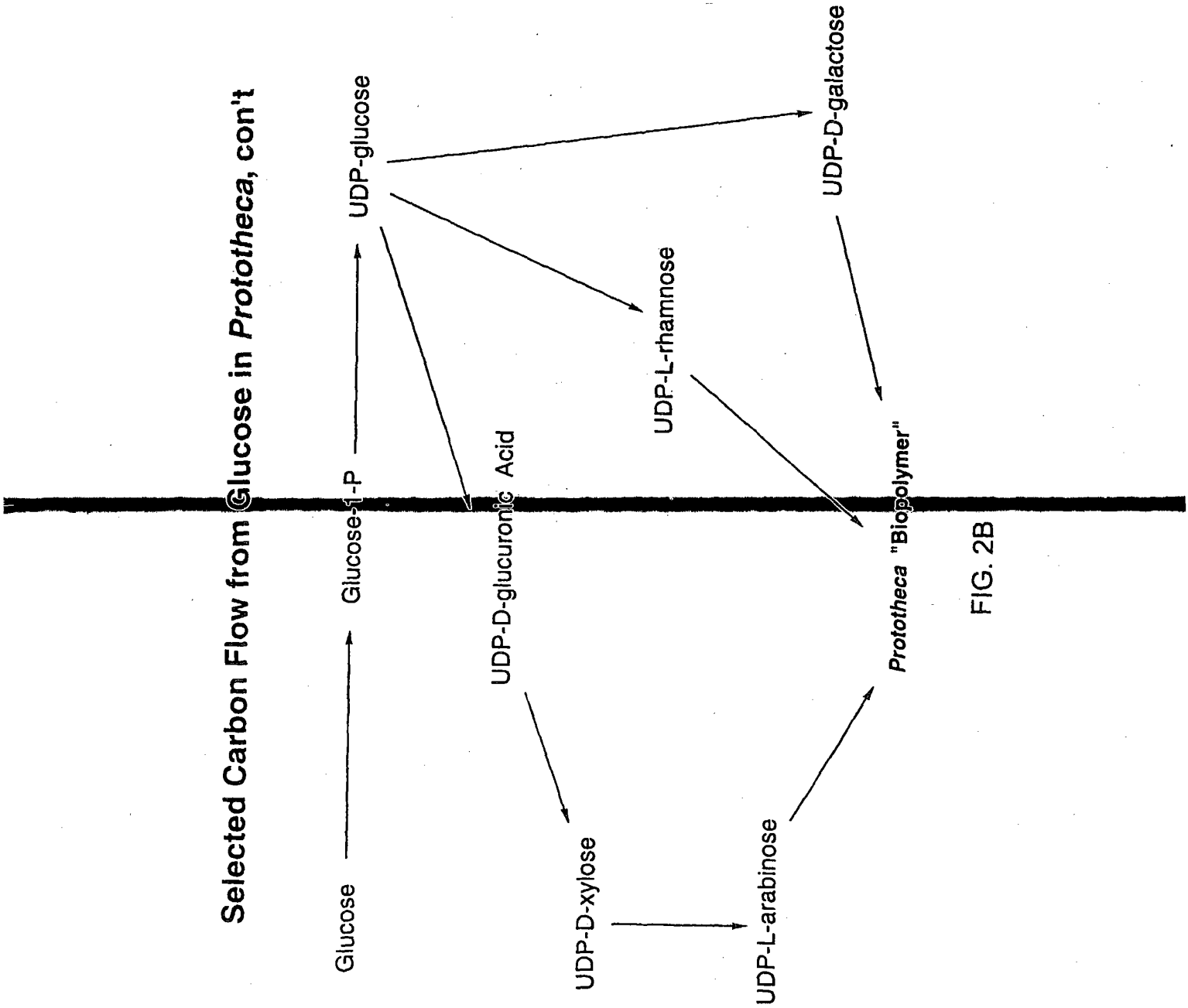
Selected Carbon Flow from Glucose in *Prototheca*, con't
Glucose
Glucose-1-P
UDP-glucose
UDP-D-glucuronic Acid
UDP-D-xylose
UDP-L-arabinose
UDP-L-rhamnose
UDP-D-galactose
Prototheca "Biopolymer"

FIG. 2B

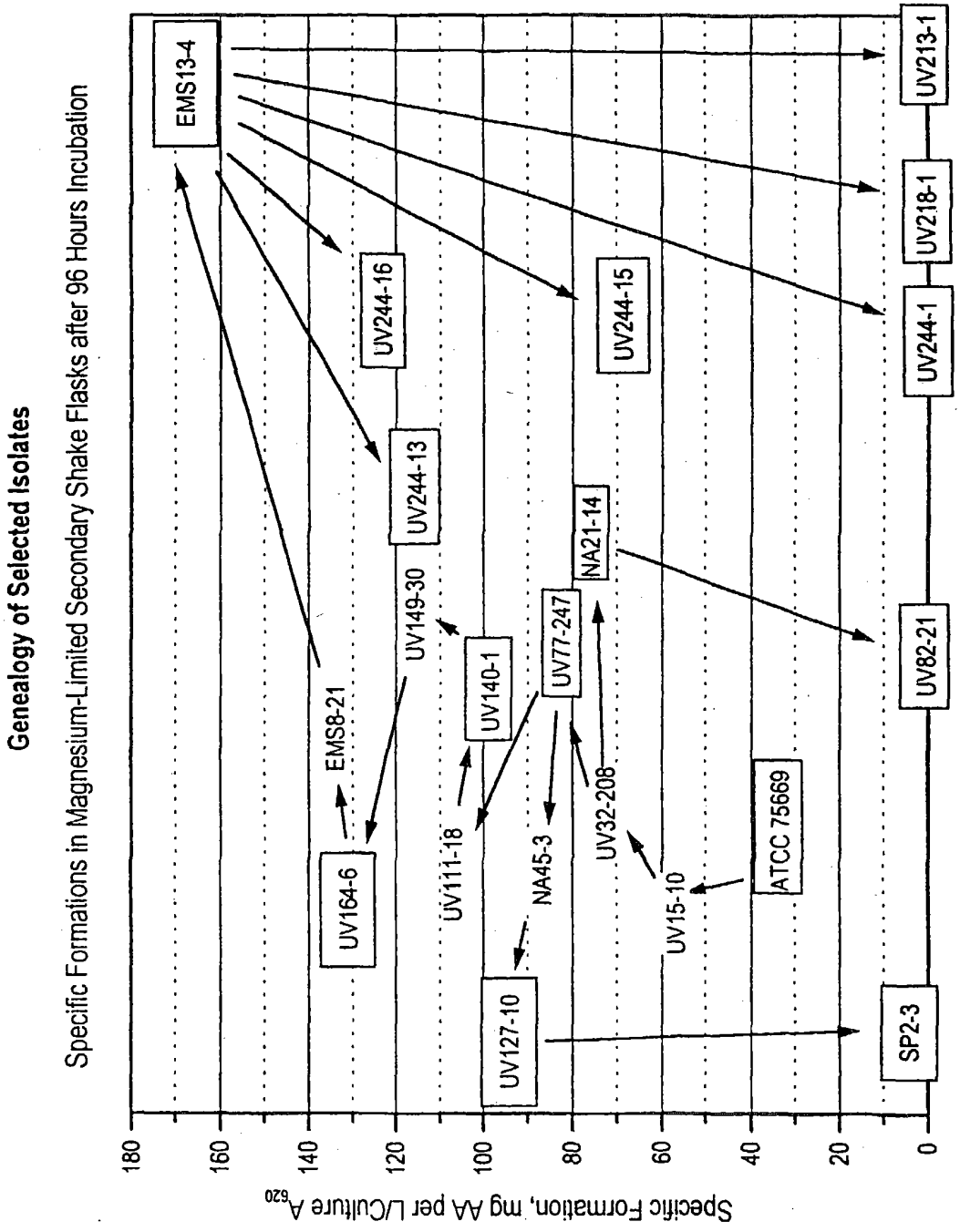

FIG. 3

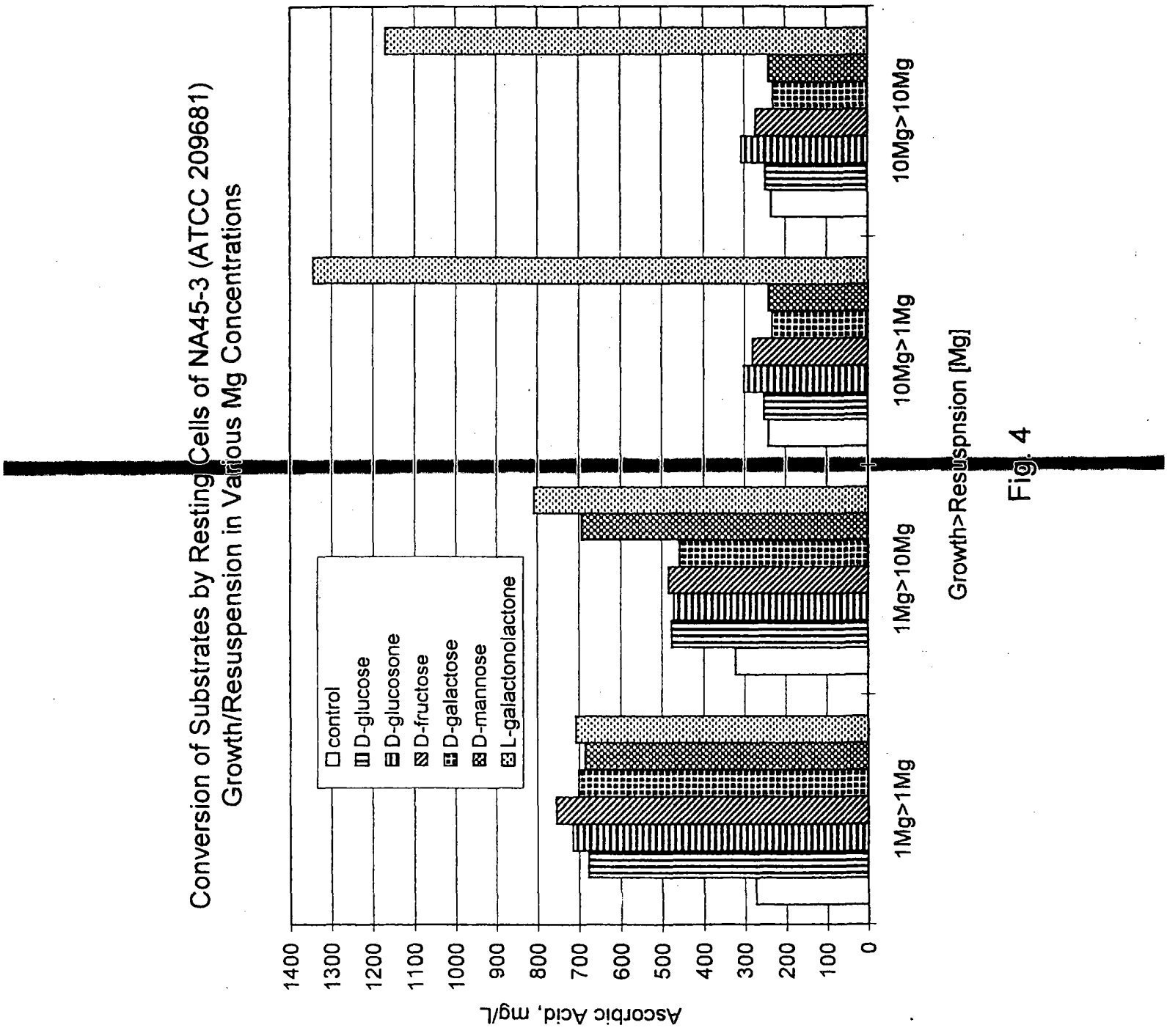
Conversion of Substrates by Resting Cells of NA45-3 (ATCC 209681)
Growth/Resuspension in Various Mg Concentrations
Ascorbic Acid, mg/L
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
control
D-glucose
D-glucosone
D-fructose
D-galactose
D-mannose
L-galactonolactone
1Mg>1Mg
1Mg>10Mg
10Mg>1Mg
10Mg>10Mg
Growth>Resuspnsion [Mg]

Fig. 4

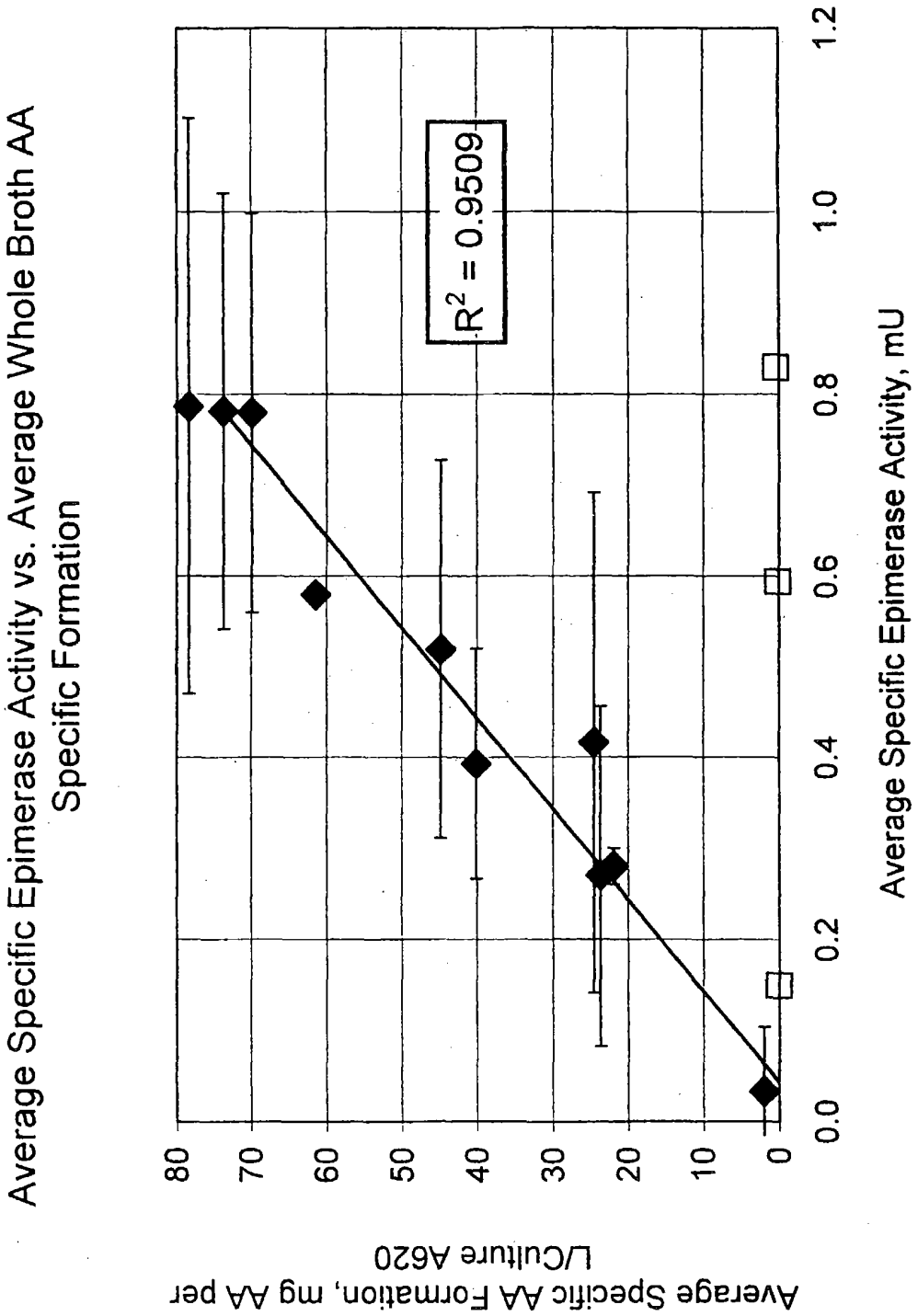

Fig. 5

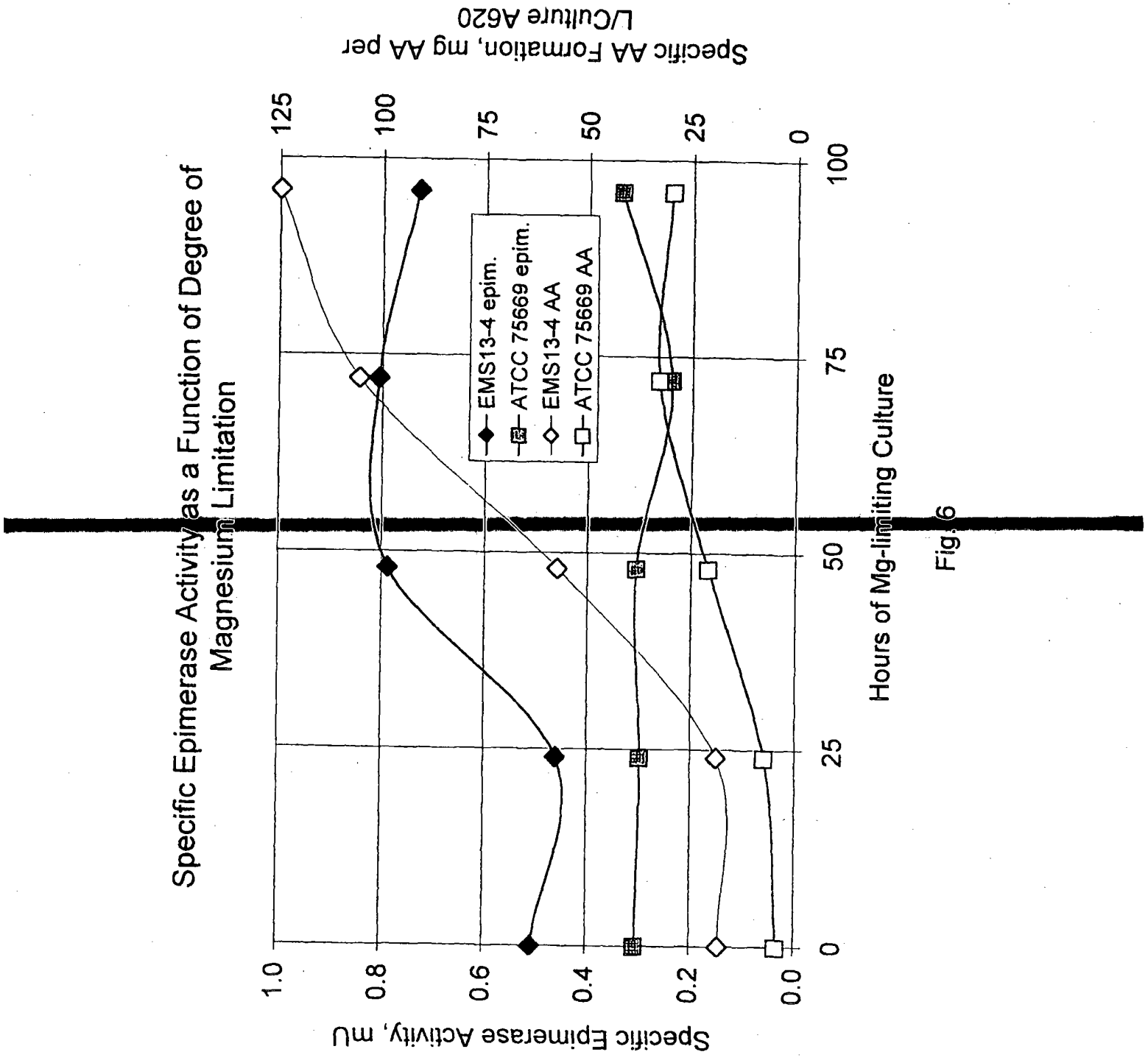
Specific Epimerase Activity as a Function of Degree of Magnesium Limitation
Specific Epimerase Activity, mU
1.0
0.8
0.6
0.4
0.2
0.0
Specific AA Formation, mg AA per L/Culture A620
125
100
75
50
25
0
Hours of Mg-limiting Culture
0
25
50
75
100
EMS13-4 epim.
ATCC 75669 epim.
EMS13-4 AA
ATCC 75669 AA

Fig. 6

Proposed Mechanism for the Conversion of GDP-D-mannose to GDP-L-galactose in *Chlorella pyrenoidosa* (Barber)

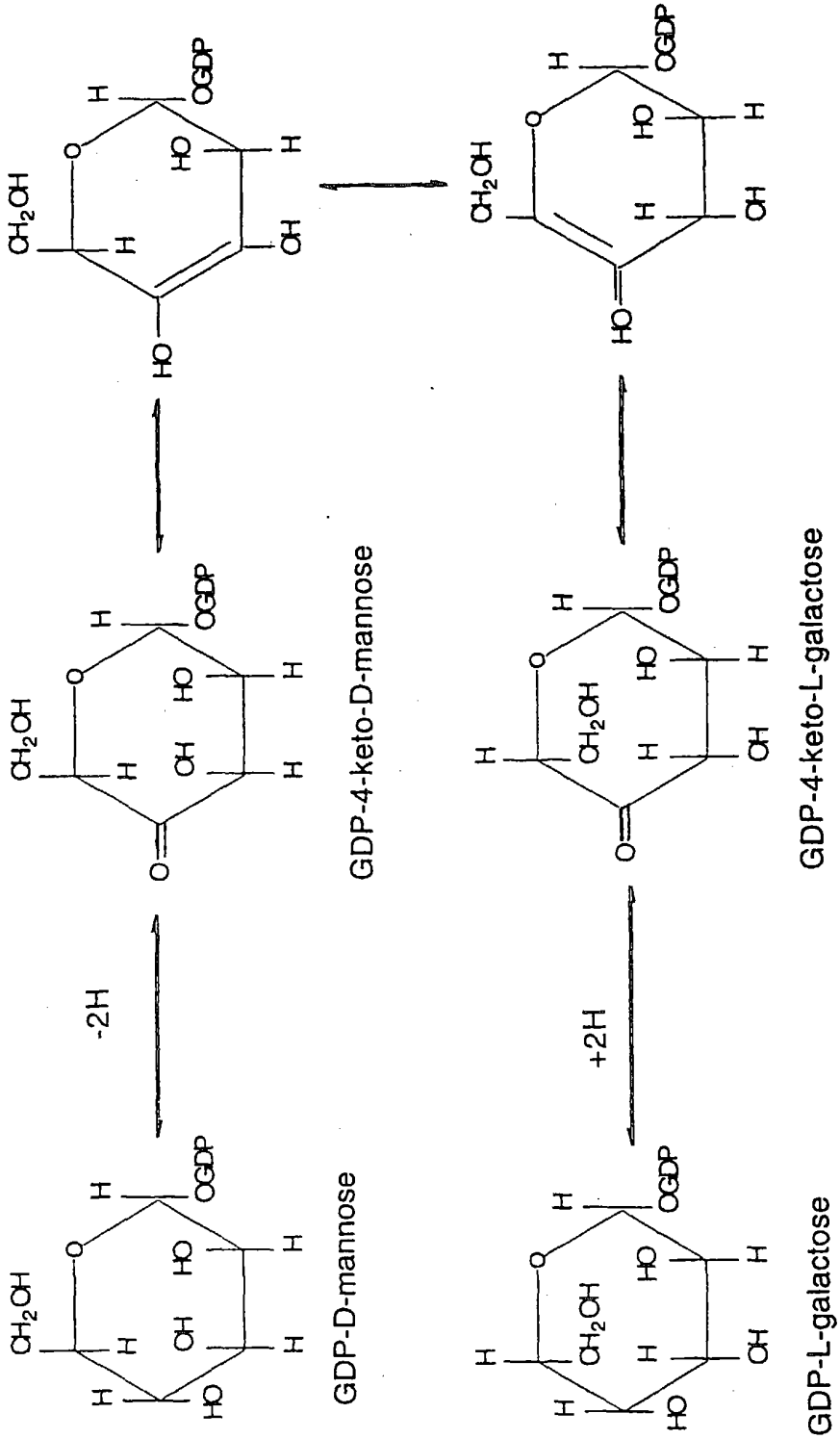

Fig. 7

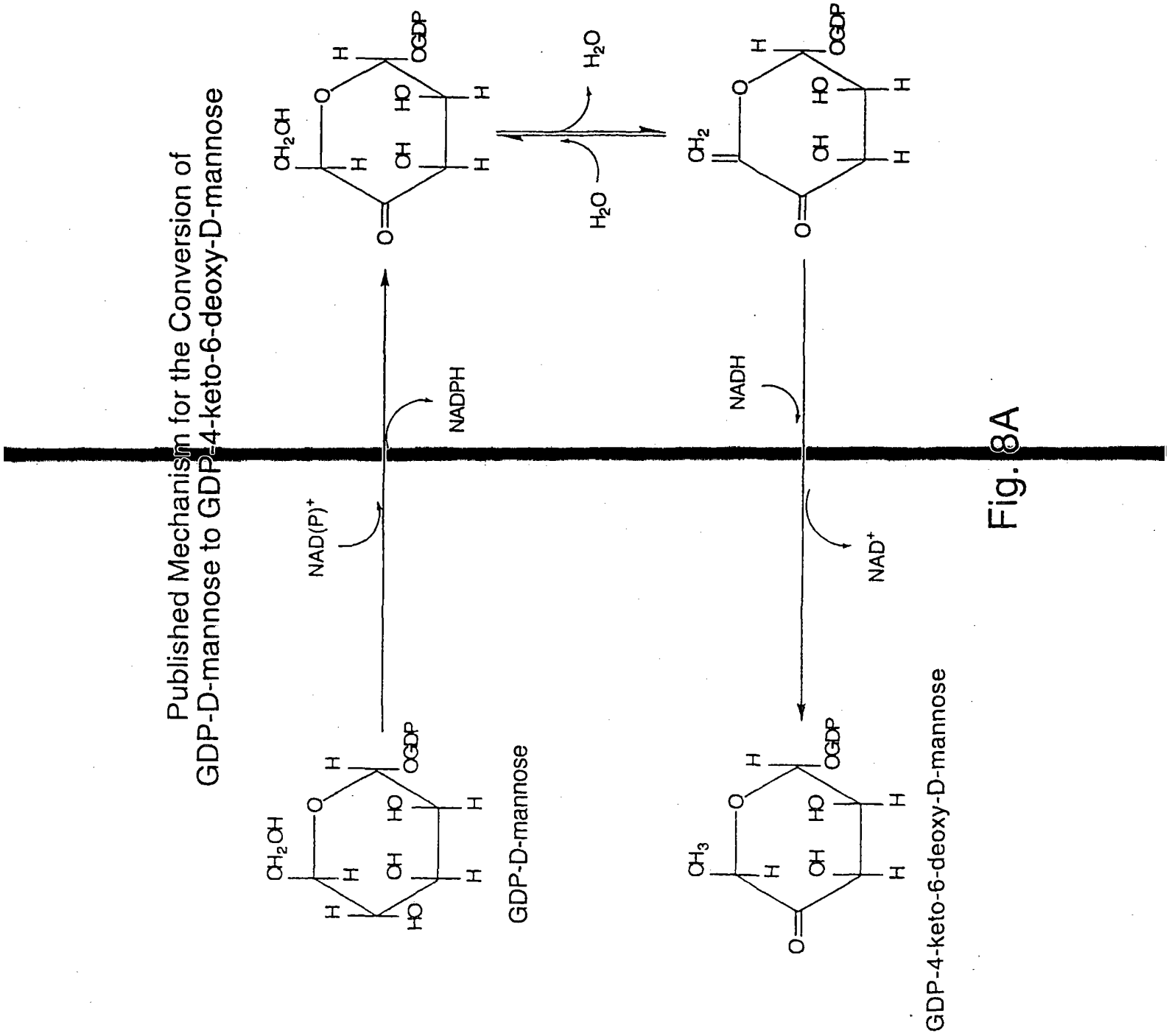
Published Mechanism for the Conversion of
GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose
NAD(P)+
NADPH
NADH
NAD+
H2O
H2O
GDP-D-mannose
GDP-4-keto-6-deoxy-D-mannose

Fig. 8A

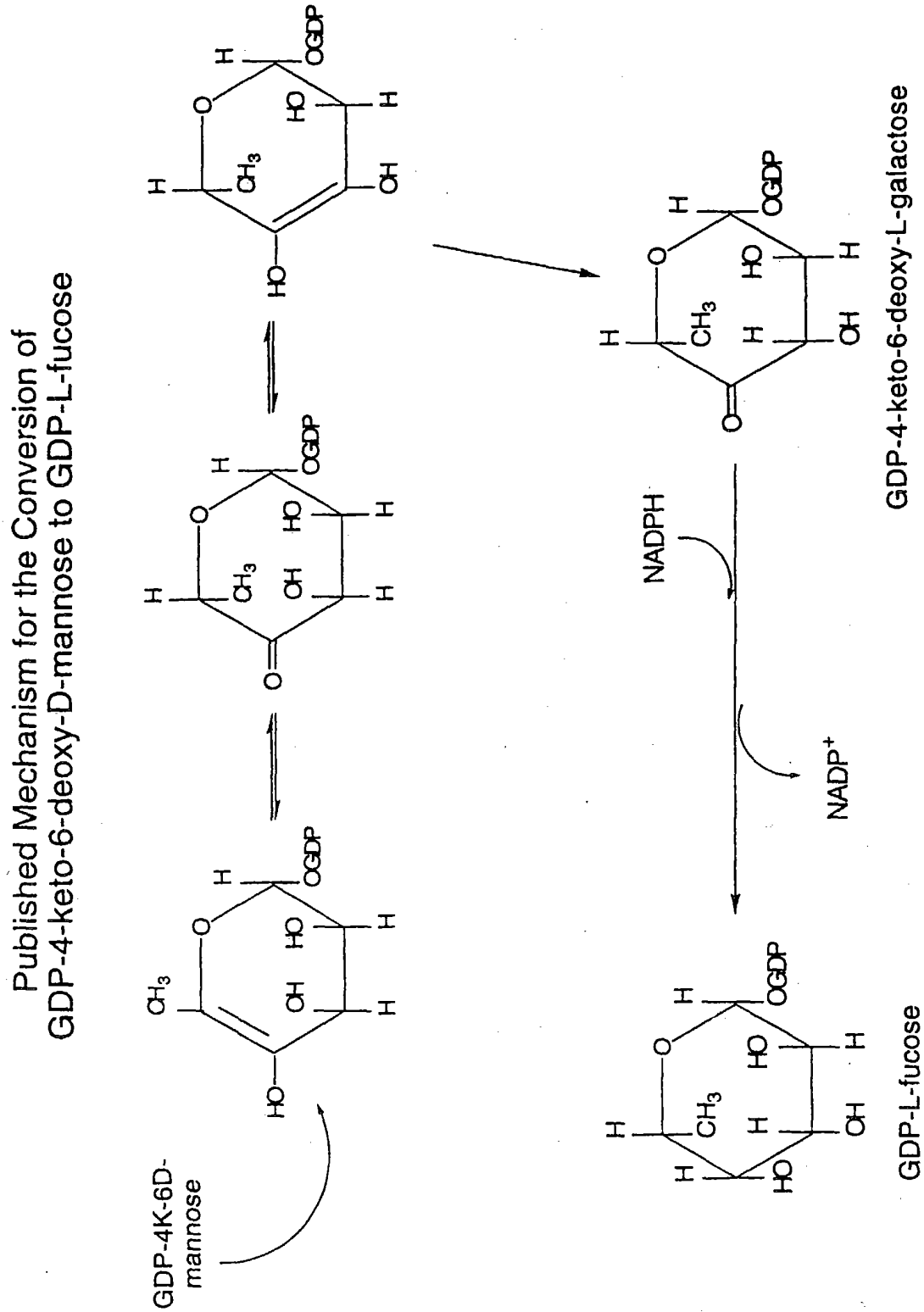

Fig. 8B

SEQUENCE LISTING

<110> Berry, Alan
Running, Jeffrey A.
Severson, David K.
Burlingame, Richard P.

<120> "VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS"

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gaa cca act aat gag tgg tat gct att gct aag atc gct ggg att aag   489
Glu Pro Thr Asn Glu Trp Tyr Ala Ile Ala Lys Ile Ala Gly Ile Lys
            135                 140                 145

act tgt cag gct tat agg att cag cac gga tgg gat gca atc tct ggc   537
Thr Cys Gln Ala Tyr Arg Ile Gln His Gly Trp Asp Ala Ile Ser Gly
        150                 155                 160

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Met Pro Thr Asn Leu Tyr Gly Pro Asn Asp Asn Phe His Pro Glu Ser
    165                 170                 175

cat gtg ctt cct gct ctt atg agg agg ttc cac gag gcg aaa gtg aat   633
His Val Leu Pro Ala Leu Met Arg Arg Phe His Glu Ala Lys Val Asn
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Gln Glu Val Thr Ile Arg Glu Leu Ala Glu Leu Val Lys Glu Val Val
    245                 250                 255

ggt ttt gaa ggg aag ctt gga tgg gat tgc act aag cca gat ggc aca   873
Gly Phe Glu Gly Lys Leu Gly Trp Asp Cys Thr Lys Pro Asp Gly Thr
260                 265                 270                 275

ccg agg aaa ctt atg gac agc tca aag ctc gcg tct ttg ggt tgg aca   921
Pro Arg Lys Leu Met Asp Ser Ser Lys Leu Ala Ser Leu Gly Trp Thr
                280                 285                 290

cct aag gtt tct ctt aga gat ggt ctg agc caa act tat gat tgg tat   969
Pro Lys Val Ser Leu Arg Asp Gly Leu Ser Gln Thr Tyr Asp Trp Tyr
            295                 300                 305

ttg aag aat gtt tgc aac cga taa gttaatggtt tctcttctca tatatacaca  1023
Leu Lys Asn Val Cys Asn Arg
        310                 315

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     50                  55                  60

Ala Ala Lys Val Gly Gly Ile His Ala Asn Asn Thr Tyr Pro Ala Asp
 65                  70                  75                  80

Phe Ile Gly Val Asn Leu Gln Ile Gln Thr Asn Val Ile His Ser Ala
                 85                  90                  95

Tyr Glu His Gly Val Lys Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile
            100                 105                 110

Tyr Pro Lys Phe Ala Pro Gln Pro Ile Pro Glu Ser Ala Leu Leu Thr
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Ala Ser Leu Glu Pro Thr Asn Glu Trp Tyr Ala Ile Ala Lys Ile Ala
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Gly Ile Lys Thr Cys Gln Ala Tyr Arg Ile Gln His Gly Trp Asp Ala
145                 150                 155                 160

Ile Ser Gly Met Pro Thr Asn Leu Tyr Gly Pro Asn Asp Asn Phe His
                165                 170                 175

Pro Glu Ser His Val Leu Pro Ala Leu Met Arg Arg Phe His Glu Ala
            180                 185                 190

Lys Val Asn Trp Ser Gly Gly Ser Cys Gly Val Gly Tyr Lys Val Val
        195                 200                 205

Pro Leu Glu Gly Lys Phe Leu His Val Asp Asp Leu Ala Asp Ala Cys
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Val Phe Leu Leu Asp Arg Ile Gln Arg Gly Leu Glu His Val Asn Ile
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Glu Val Val Gly Phe Glu Gly Lys Leu Gly Trp Asp Cys Thr Lys Pro
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Asp Gly Thr Pro Arg Lys Leu Met Asp Ser Ser Lys Leu Ala Ser Leu
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             20                  25                  30

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         35                  40                  45

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     50                  55                  60

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Lys Val Gly Gly Ile Val Ala Asn Asn Thr Tyr Pro Ala Asp Phe Ile
 65                  70                  75                  80

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Asn Asp Val Asn Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile Tyr Pro
            100                 105                 110

aaa ctg gca aaa cag ccg atg gca gaa agc gag ttg ttg cag ggc acg   384
Lys Leu Ala Lys Gln Pro Met Ala Glu Ser Glu Leu Leu Gln Gly Thr
        115                 120                 125

ctg gag ccg act aac gag cct tat gct att gcc aaa atc gcc ggg atc   432
Leu Glu Pro Thr Asn Glu Pro Tyr Ala Ile Ala Lys Ile Ala Gly Ile
    130                 135                 140

aaa ctg tgc gaa tca tac aac cgc cag tac gga cgc gat tac cgc tca   480
Lys Leu Cys Glu Ser Tyr Asn Arg Gln Tyr Gly Arg Asp Tyr Arg Ser
145                 150                 155                 160

gtc atg ccg acc aac ctg tac ggg cca cac gac aac ttc cac ccg agt   528
Val Met Pro Thr Asn Leu Tyr Gly Pro His Asp Asn Phe His Pro Ser
                165                 170                 175

aat tcg cat gtg atc cca gca ttg ctg cgt cgc ttc cac gag gcg acg   576
Asn Ser His Val Ile Pro Ala Leu Leu Arg Arg Phe His Glu Ala Thr
            180                 185                 190

gca cag aat gcg ccg gac gtg gtg gta tgg ggc agc ggt aca ccg atg   624
Ala Gln Asn Ala Pro Asp Val Val Val Trp Gly Ser Gly Thr Pro Met
        195                 200                 205

cgc gaa ttt ctg cac gtc gat gat atg gcg gcg gcg agc att cat gtc   672
Arg Glu Phe Leu His Val Asp Asp Met Ala Ala Ala Ser Ile His Val
    210                 215                 220

atg gag ctg gcg cat gaa gtc tgg ctg gag aac acc cag ccg atg ttg   720
Met Glu Leu Ala His Glu Val Trp Leu Glu Asn Thr Gln Pro Met Leu
225                 230                 235                 240

tcg cac att aac gtc ggc acg ggc gtt gac tgc act atc cgc gac gtg   768
Ser His Ile Asn Val Gly Thr Gly Val Asp Cys Thr Ile Arg Asp Val
                245                 250                 255

gcg caa acc atc gcc aaa gtg gtg ggt tac aaa ggc cgg gtg gtt ttt   816
Ala Gln Thr Ile Ala Lys Val Val Gly Tyr Lys Gly Arg Val Val Phe
            260                 265                 270

gat gcc agc aaa ccg gat ggc acg ccg cgc aaa ctg ctg gat gtg acg   864
```

```
Asp Ala Ser Lys Pro Asp Gly Thr Pro Arg Lys Leu Leu Asp Val Thr
        275                 280                 285

cgc ctg cat cag ctt ggc tgg tat cac gaa atc tca ctg gaa gcg ggg   912
Arg Leu His Gln Leu Gly Trp Tyr His Glu Ile Ser L u Glu Ala Gly
    290                 295                 300

ctt gcc agc act tac cag tgg ttc ctt gag aat caa gac cgc ttt cgg   960
Leu Ala Ser Thr Tyr Gln Trp Phe Leu Glu Asn Gln Asp Arg Phe Arg
305                 310                 315                 320

ggg taa                                                          966
Gly
```

<210> 4
<211> 321
<212> PRT
<213> Escherichia coli

<400> 4

```
Met Ser Lys Gln Arg Val Phe Ile Ala Gly His Arg Gly Met Val Gly
  1               5                  10                  15

Ser Ala Ile Arg Arg Gln Leu Glu Gln Arg Gly Asp Val Glu Leu Val
             20                  25                  30

Leu Arg Thr Arg Asp Glu Leu Asn Leu Leu Asp Ser Arg Ala Val His
         35                  40                  45

Asp Phe Phe Ala Ser Glu Arg Ile Asp Gln Val Tyr Leu Ala Ala Ala
     50                  55                  60

Lys Val Gly Gly Ile Val Ala Asn Asn Thr Tyr Pro Ala Asp Phe Ile
 65                  70                  75                  80

Tyr Gln Asn Met Met Ile Glu Ser Asn Ile Ile His Ala Ala His Gln
                 85                  90                  95

Asn Asp Val Asn Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile Tyr Pro
            100                 105                 110

Lys Leu Ala Lys Gln Pro Met Ala Glu Ser Glu Leu Leu Gln Gly Thr
        115                 120                 125

Leu Glu Pro Thr Asn Glu Pro Tyr Ala Ile Ala Lys Ile Ala Gly Ile
    130                 135                 140
```

```
Lys Leu Cys Glu Ser Tyr Asn Arg Gln Tyr Gly Arg Asp Tyr Arg S r
145                 150                 155                 160

Val Met Pro Thr Asn Leu Tyr Gly Pro His Asp Asn Phe His Pro Ser
                165                 170                 175

Asn Ser His Val Ile Pro Ala Leu Leu Arg Arg Phe His Glu Ala Thr
            180                 185                 190

Ala Gln Asn Ala Pro Asp Val Val Val Trp Gly Ser Gly Thr Pro Met
        195                 200                 205

Arg Glu Phe Leu His Val Asp Asp Met Ala Ala Ala Ser Ile His Val
    210                 215                 220

Met Glu Leu Ala His Glu Val Trp Leu Glu Asn Thr Gln Pro Met Leu
225                 230                 235                 240

Ser His Ile Asn Val Gly Thr Gly Val Asp Cys Thr Ile Arg Asp Val
                245                 250                 255

Ala Gln Thr Ile Ala Lys Val Val Gly Tyr Lys Gly Arg Val Val Phe
            260                 265                 270

Asp Ala Ser Lys Pro Asp Gly Thr Pro Arg Lys Leu Leu Asp Val Thr
        275                 280                 285

Arg Leu His Gln Leu Gly Trp Tyr His Glu Ile Ser Leu Glu Ala Gly
    290                 295                 300

Leu Ala Ser Thr Tyr Gln Trp Phe Leu Glu Asn Gln Asp Arg Phe Arg
305                 310                 315                 320

Gly
```

<210> 5
<211> 1340
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (75)..(1040)

<400> 5
ctagaattca gcggccgctg aattctagct agaattcagc ggccgctgaa ttctagaacc 60

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caggtgcaac tgac atg ggt gaa ccc cag gga tcc atg cgg att cta gtg   110
                Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val
                  1               5                  10

aca ggg ggc tct ggg ctg gta ggc aaa gcc atc cag aag gtg gta gca   158
Thr Gly Gly Ser Gly Leu Val Gly Lys Ala Ile Gln Lys Val Val Ala
         15                  20                  25

gat gga gct gga ctt cct gga gag gac tgg gtg ttt gtc tcc tct aaa   206
Asp Gly Ala Gly Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys
     30                  35                  40

gac gcc gat ctc acg gat aca gca cag acc cgc gcc ctg ttt gag aag   254
Asp Ala Asp Leu Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys
 45                  50                  55                  60

gtc caa ccc aca cac gtc atc cat ctt gct gca atg gtg ggg ggc ctg   302
Val Gln Pro Thr His Val Ile His Leu Ala Ala Met Val Gly Gly Leu
                65                  70                  75

ttc cgg aat atc aaa tac aat ttg gac ttc tgg agg aaa aac gtg cac   350
Phe Arg Asn Ile Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His
             80                  85                  90

atg aac gac aac gtc ctg cac tcg gcc ttt gag gtg ggg gcc cgc aag   398
Met Asn Asp Asn Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys
         95                 100                 105

gtg gtg tcc tgc ctg tcc acc tgt atc ttc cct gac aag acg acc tac   446
Val Val Ser Cys Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr
    110                 115                 120

ccg ata gat gag acc atg atc cac aat ggg cct ccc cac aac agc aat   494
Pro Ile Asp Glu Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn
125                 130                 135                 140

ttt ggg tac tcg tat gcc aag agg atg atc gac gtg cag aac agg gcc   542
Phe Gly Tyr Ser Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala
                145                 150                 155

tac ttc cag cag tac ggc tgc acc ttc acc gct gtc atc ccc acc aac   590
Tyr Phe Gln Gln Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn
            160                 165                 170

gtt ttc ggg ccc cac gac aac ttc aac atc gag gat ggc cac gtg ctg   638
Val Phe Gly Pro His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu
        175                 180                 185
```

```
cct ggc ctc atc cac aag gtg cac ctg gcc aag agc agc ggc tcg gcc   686
Pro Gly Leu Ile His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala
    190                 195                 200

ctg acg gtg tgg ggt aca ggg aat ccg cgg agg cag ttc ata tac tcg   734
Leu Thr Val Trp Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser
205                 210                 215                 220

ctg gac ctg gcc cag ctc ttt atc tgg gtc ctg cgg gag tac aat gaa   782
Leu Asp Leu Ala Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu
                225                 230                 235

gtg gag ccc atc atc ctc tcc gtg ggc gag gaa gat gag gtc tcc atc   830
Val Glu Pro Ile Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile
            240                 245                 250

aag gag gca gcc gag gcg gtg gtg gag gcc atg gac ttc cat ggg gaa   878
Lys Glu Ala Ala Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu
        255                 260                 265

gtc acc ttt gat aca acc aag tcg gat ggg cag ttt aag aag aca gcc   926
Val Thr Phe Asp Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala
    270                 275                 280

agt aac agc aag ctg agg acc tac ctg ccc gac ttc cgg ttc aca ccc   974
Ser Asn Ser Lys Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro
285                 290                 295                 300

ttc aag cag gcg gtg aag gag acc tgt gct tgg ttc act gac aac tac   1022
Phe Lys Gln Ala Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr
                305                 310                 315

gag cag gcc cgg aag tga agctggaaga caggatcagg tgccagcgga          1070
Glu Gln Ala Arg Lys
            320

ccatcggctg gcagagccca gcggccacca cccgtcaacc ctgccaggag ctgagggcac 1130

cacccagcaa cctgggcctg cattccatcc gctctgcagc cccaagcatc tttccagtgg 1190

ggcccccatt cacgttggtc ctcagggaaa ccagggtccg gggcaggccc ggcgctttgc 1250

tccccacacc agcccccctgc gcgtgtccac tctgatcctg catcccactc cctgggagcc 1310

aataaagtgc attttcacag aaaaaaaaaa                                   1340
```

<210> 6

<211> 321
<212> PRT
<213> Homo sapiens

<400> 6

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Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val Thr Gly Gly Ser
 1               5                  10                  15

Gly Leu Val Gly Lys Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly
            20                  25                  30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu
        35                  40                  45

Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys Val Gln Pro Thr
    50                  55                  60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile
65                  70                  75                  80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Met Asn Asp Asn
                85                  90                  95

Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys Val Val Ser Cys
            100                 105                 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu
        115                 120                 125

Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn Phe Gly Tyr Ser
    130                 135                 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln
145                 150                 155                 160

Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro
                165                 170                 175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile
            180                 185                 190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp
        195                 200                 205

Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala
    210                 215                 220

Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile
```

225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala
245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu Val Thr Phe Asp
260 265 270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Ser Lys
275 280 285

Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala
290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg
305 310 315 320

Lys

<210> 7
<211> 1017
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(1017)

<400> 7
atg aga gtt ctg gtt acc ggt ggt agc ggt tac att gga agt cat acc 48
Met Arg Val Leu Val Thr Gly Gly Ser Gly Tyr Ile Gly Ser His Thr
1 5 10 15

tgt gtg caa tta ctg caa aac ggt cat gat gtc atc att ctt gat aac 96
Cys Val Gln Leu Leu Gln Asn Gly His Asp Val Ile Ile Leu Asp Asn
20 25 30

ctc tgt aac agt aag cgc agc gta ctg cct gtt atc gag cgt tta ggc 144
Leu Cys Asn Ser Lys Arg Ser Val Leu Pro Val Ile Glu Arg Leu Gly
35 40 45

ggc aaa cat cca acg ttt gtt gaa ggc gat att cgt aac gaa gcg ttg 192
Gly Lys His Pro Thr Phe Val Glu Gly Asp Ile Arg Asn Glu Ala Leu
50 55 60

atg acc gag atc ctg cac gat cac gct atc gac acc gtg atc cac ttc 240
Met Thr Glu Ile Leu His Asp His Ala Ile Asp Thr Val Ile His Phe

```
65                  70                  75                  80

gcc ggg ctg aaa gcc gtg ggc gaa tcg gta caa aaa ccg ctg gaa tat   288
Ala Gly Leu Lys Ala Val Gly Glu Ser Val Gln Lys Pro Leu Glu Tyr
                85                  90                  95

tac gac aac aat gtc aac ggc act ctg cgc ctg att agc gcc atg cgc   336
Tyr Asp Asn Asn Val Asn Gly Thr Leu Arg Leu Ile Ser Ala Met Arg
            100                 105                 110

gcc gct aac gtc aaa aac ttt att ttt agc tcc tcc gcc acc gtt tat   384
Ala Ala Asn Val Lys Asn Phe Ile Phe Ser Ser Ser Ala Thr Val Tyr
        115                 120                 125

ggc gat cag ccc aaa att cca tac gtt gaa agc ttc ccg acc ggc aca   432
Gly Asp Gln Pro Lys Ile Pro Tyr Val Glu Ser Phe Pro Thr Gly Thr
    130                 135                 140

ccg caa agc cct tac ggc aaa agc aag ctg atg gtg gaa cag atc ctc   480
Pro Gln Ser Pro Tyr Gly Lys Ser Lys Leu Met Val Glu Gln Ile Leu
145                 150                 155                 160

acc gat ctg caa aaa gcc cag ccg gac tgg agc att gcc ctg ctg cgc   528
Thr Asp Leu Gln Lys Ala Gln Pro Asp Trp Ser Ile Ala Leu Leu Arg
                165                 170                 175

tac ttc aac ccg gtt ggc gcg cat ccg tcg ggc gat atg ggc gaa gat   576
Tyr Phe Asn Pro Val Gly Ala His Pro Ser Gly Asp Met Gly Glu Asp
            180                 185                 190

ccg caa ggc att ccg aat aac ctg atg cca tac atc gcc cag gtt gct   624
Pro Gln Gly Ile Pro Asn Asn Leu Met Pro Tyr Ile Ala Gln Val Ala
        195                 200                 205

gta ggc cgt cgc gac tcg ctg gcg att ttt ggt aac gat tat ccg acc   672
Val Gly Arg Arg Asp Ser Leu Ala Ile Phe Gly Asn Asp Tyr Pro Thr
    210                 215                 220

gaa gat ggt act ggc gta cgc gat tac atc cac gta atg gat ctg gcg   720
Glu Asp Gly Thr Gly Val Arg Asp Tyr Ile His Val Met Asp Leu Ala
225                 230                 235                 240

gac ggt cac gtc gtg gcg atg gaa aaa ctg gcg aac aag cca ggc gta   768
Asp Gly His Val Val Ala Met Glu Lys Leu Ala Asn Lys Pro Gly Val
                245                 250                 255

cac atc tac aac ctc ggc gct ggc gta ggc aac agc gtg ctg gac gtg   816
His Ile Tyr Asn Leu Gly Ala Gly Val Gly Asn Ser Val Leu Asp Val
```

```
            260                 265                 270

gtt aat gcc ttc agc aaa gcc tgc ggc aaa ccg gtt aat tat cat ttt   864
Val Asn Ala Phe Ser Lys Ala Cys Gly Lys Pro Val Asn Tyr His Phe
        275                 280                 285

gca ccg cgt cgc gag ggc gac ctt ccg gcc tac tgg gcg gac gcc agc   912
Ala Pro Arg Arg Glu Gly Asp Leu Pro Ala Tyr Trp Ala Asp Ala Ser
    290                 295                 300

aaa gcc gac cgt gaa ctg aac tgg cgc gta acg cgc aca ctc gat gaa   960
Lys Ala Asp Arg Glu Leu Asn Trp Arg Val Thr Arg Thr Leu Asp Glu
305                 310                 315                 320

atg gcg cag gac acc tgg cac tgg cag tca cgc cat cca cag gga tat   1008
Met Ala Gln Asp Thr Trp His Trp Gln Ser Arg His Pro Gln Gly Tyr
                325                 330                 335

ccc gat taa                                                       1017
Pro Asp
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<210> 8
<211> 338
<212> PRT
<213> Escherichia coli

<400> 8

```
Met Arg Val Leu Val Thr Gly Gly Ser Gly Tyr Ile Gly Ser His Thr
  1               5                  10                  15

Cys Val Gln Leu Leu Gln Asn Gly His Asp Val Ile Ile Leu Asp Asn
             20                  25                  30

Leu Cys Asn Ser Lys Arg Ser Val Leu Pro Val Ile Glu Arg Leu Gly
         35                  40                  45

Gly Lys His Pro Thr Phe Val Glu Gly Asp Ile Arg Asn Glu Ala Leu
     50                  55                  60

Met Thr Glu Ile Leu His Asp His Ala Ile Asp Thr Val Ile His Phe
 65                  70                  75                  80

Ala Gly Leu Lys Ala Val Gly Glu Ser Val Gln Lys Pro Leu Glu Tyr
                 85                  90                  95

Tyr Asp Asn Asn Val Asn Gly Thr Leu Arg Leu Ile Ser Ala Met Arg
            100                 105                 110
```

```
Ala Ala Asn Val Lys Asn Phe Ile Phe Ser Ser Ser Ala Thr Val Tyr
        115                 120                 125

Gly Asp Gln Pro Lys Ile Pro Tyr Val Glu Ser Phe Pro Thr Gly Thr
    130                 135                 140

Pro Gln Ser Pro Tyr Gly Lys Ser Lys Leu Met Val Glu Gln Ile Leu
145                 150                 155                 160

Thr Asp Leu Gln Lys Ala Gln Pro Asp Trp Ser Ile Ala Leu Leu Arg
                165                 170                 175

Tyr Phe Asn Pro Val Gly Ala His Pro Ser Gly Asp Met Gly Glu Asp
            180                 185                 190

Pro Gln Gly Ile Pro Asn Asn Leu Met Pro Tyr Ile Ala Gln Val Ala
        195                 200                 205

Val Gly Arg Arg Asp Ser Leu Ala Ile Phe Gly Asn Asp Tyr Pro Thr
    210                 215                 220

Glu Asp Gly Thr Gly Val Arg Asp Tyr Ile His Val Met Asp Leu Ala
225                 230                 235                 240

Asp Gly His Val Val Ala Met Glu Lys Leu Ala Asn Lys Pro Gly Val
                245                 250                 255

His Ile Tyr Asn Leu Gly Ala Gly Val Gly Asn Ser Val Leu Asp Val
            260                 265                 270

Val Asn Ala Phe Ser Lys Ala Cys Gly Lys Pro Val Asn Tyr His Phe
        275                 280                 285

Ala Pro Arg Arg Glu Gly Asp Leu Pro Ala Tyr Trp Ala Asp Ala Ser
    290                 295                 300

Lys Ala Asp Arg Glu Leu Asn Trp Arg Val Thr Arg Thr Leu Asp Glu
305                 310                 315                 320

Met Ala Gln Asp Thr Trp His Trp Gln Ser Arg His Pro Gln Gly Tyr
                325                 330                 335

Pro Asp
```

<210> 9
<211> 1047

<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1047)

<400> 9

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atg gca gag aag gtg ctg gta aca ggt ggg gct ggc tac att ggc agc   48
Met Ala Glu Lys Val Leu Val Thr Gly Gly Ala Gly Tyr Ile Gly Ser
  1               5                  10                  15

cac acg gtg ctg gag ctg ctg gag gct ggc tac ttg cct gtg gtc atc   96
His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
             20                  25                  30

gat aac ttc cat aat gcc ttc cgt gga ggg ggc tcc ctg cct gag agc   144
Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
         35                  40                  45

ctg cgg cgg gtc cag gag ctg aca ggc cgc tct gtg gag ttt gag gag   192
Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
     50                  55                  60

atg gac att ttg gac cag gga gcc cta cag cgt ctc ttc aaa aag tac   240
Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
 65                  70                  75                  80

agc ttt atg gcg gtc atc cac ttt gcg ggg ctc aag gcc gtg ggc gag   288
Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
                 85                  90                  95

tcg gtg cag aag cct ctg gat tat tac aga gtt aac ctg acc ggg acc   336
Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
            100                 105                 110

atc cag ctt ctg gag atc atg aag gcc cac ggg gtg aag aac ctg gtg   384
Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
        115                 120                 125

ttc agc agc tca gcc act gtg tac ggg aac ccc cag tac ctg ccc ctt   432
Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
    130                 135                 140

gat gag gcc cac ccc acg ggt ggt tgt acc aac cct tac ggc aag tcc   480
Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145                 150                 155                 160
```

```
aag ttc ttc atc gag gaa atg atc cgg gac ctg tgc cag gca gac aag   528
Lys Phe Phe Il  Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
                165                 170                 175

act tgg aac gta gtg ctg ctg cgc tat ttc aac ccc aca ggt gcc cat   576
Thr Trp Asn Val Val Leu Leu Arg Tyr Ph  Asn Pro Thr Gly Ala His
            180                 185                 190

gcc tct ggc tgc att ggt gag gat ccc cag ggc ata ccc aac aac ctc   624
Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
        195                 200                 205

atg cct tat gtc tcc cag gtg gcg atc ggg cga cgg gag gcc ctg aat   672
Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
    210                 215                 220

gtc ttt ggc aat gac tat gac aca gag gat ggc aca ggt gtc cgg gat   720
Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
225                 230                 235                 240

tac atc cat gtc gtg gat ctg gcc aag ggc cac att gca gcc tta agg   768
Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
                245                 250                 255

aag ctg aaa gaa cag tgt ggc tgc cgg atc tac aac ctg ggc acg ggc   816
Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
            260                 265                 270

aca ggc tat tca gtg ctg cag atg gtc cag gct atg gag aag gcc tct   864
Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
        275                 280                 285

ggg aag aag atc ccg tac aag gtg gtg gca cgg cgg gaa ggt gat gtg   912
Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
    290                 295                 300

gca gcc tgt tac gcc aac ccc agc ctg gcc caa gag gag ctg ggg tgg   960
Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305                 310                 315                 320

aca gca gcc tta ggg ctg gac agg atg tgt gag gat ctc tgg cgc tgg   1008
Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
                325                 330                 335

cag aag cag aat cct tca ggc ttt ggc acg caa gcc tga               1047
Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
            340                 345
```

<210> 10
<211> 348
<212> PRT
<213> Homo sapiens

<400> 10

```
Met Ala Glu Lys Val Leu Val Thr Gly Gly Ala Gly Tyr Ile Gly Ser
  1               5                  10                  15

His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
             20                  25                  30

Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
         35                  40                  45

Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
     50                  55                  60

Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
 65                  70                  75                  80

Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
                 85                  90                  95

Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
            100                 105                 110

Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
        115                 120                 125

Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
    130                 135                 140

Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145                 150                 155                 160

Lys Phe Phe Ile Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
                165                 170                 175

Thr Trp Asn Val Val Leu Leu Arg Tyr Phe Asn Pro Thr Gly Ala His
            180                 185                 190

Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
        195                 200                 205

Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
    210                 215                 220
```

```
Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
225                 230                 235                 240

Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
                245                 250                 255

Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
            260                 265                 270

Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
        275                 280                 285

Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
    290                 295                 300

Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305                 310                 315                 320

Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
                325                 330                 335

Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
            340                 345
```

<210> 11
<211> 317
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:CONSENSUS

<400> 11

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Xaa Xaa Arg Xaa Xaa Xaa Xaa Gly Xaa Xaa Gly Xaa Xaa Gly Xaa Xaa
  1               5                  10                  15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
             20                  25                  30

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12P 19/00, 17/04; C12N 1/12, 1/20, 5/00, 5/04
US CL :435/72, 126, 252.1, 252.3, 410, 419

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/72, 126, 252.1, 252.3, 410, 419

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, SCISEARCH, BIOTECHDS, NTIS, WPIDS, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 85/01745 A1 (KRAFT, INC.) 25 April 1985 (23.04.85), see the entire document specially ages 4-7.	1-72
Y	NIKISHIMI et al. Occupance in Yeast of L-Galactonolactone Oxidase which is similar to a key enzyme for Ascorbic Acid biosynthesis in animals, L-Gulonolactone Oxidase. Arch. Biocem. Biophys. December 1978, Vol. 191, No. 2, pages 479-486, see the entire article, specially abstract and introduction sections.	1-72
A,P	WO 99/33995 A1 (ASCORBX LIMITED) 08 July 1999 (08.07.99), see the entire article.	1-72

☐ *Further documents are listed in the continuation of Box C.* ☐ *See patent family annex.*

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
23 AUGUST 1999	22 OCT 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MARYAM MONSHIPOURI
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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CHEMICAL MATRIX

Form PCT/ISA/210 (second sheet)(July 1992)*

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This international Preliminary Examining Authority has found 2 inventions claimed in the International application covered by the claims indicated below:

Group I, claims 1-59 and 71, drawn to a method of producing ascorbic acid or esters thereof in a microorganism comprising culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase etc. as well as a microorganism genetically modified for producing ascorbic acid.

Group II, claims 60-70 and 72, drawn to a plant for producing ascorbic acid or esters thereof, wherein said plant has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase etc.

The inventions listed as Groups I-II do not relate to a single inventive concept because they are considered to be two different categories of invention and are not drawn to combination of categories (i.e. categories 1-5), specified in 37 CFR section 1.475(b).